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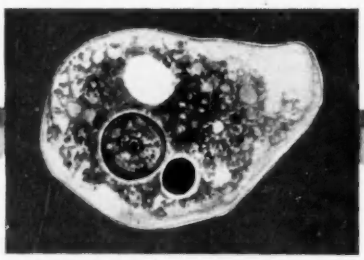

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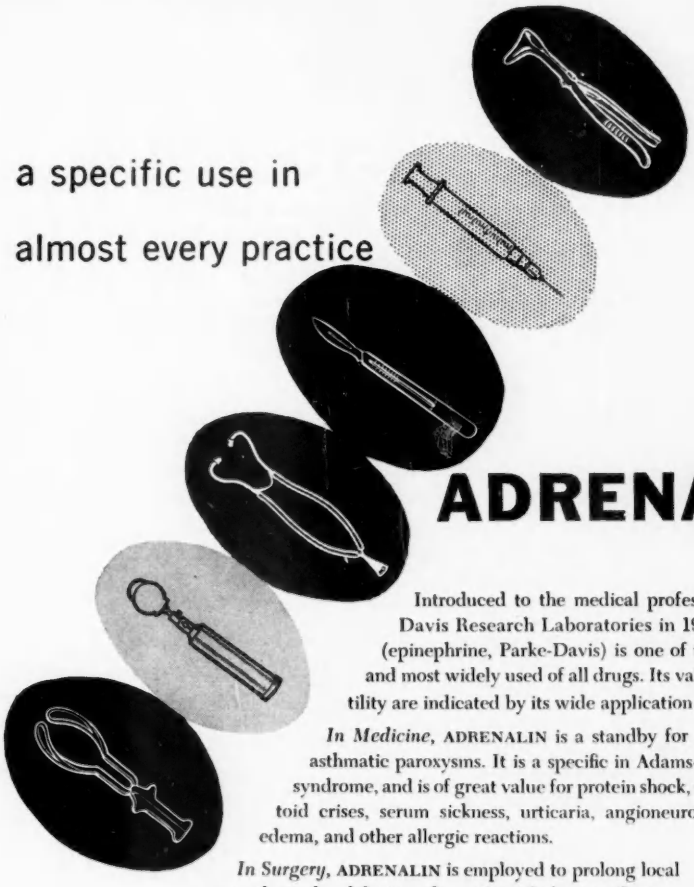

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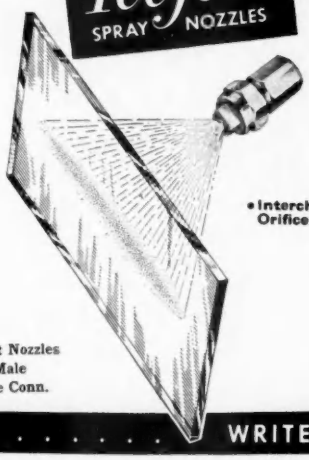

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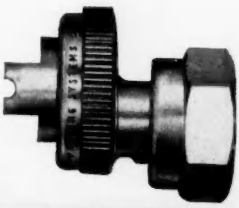

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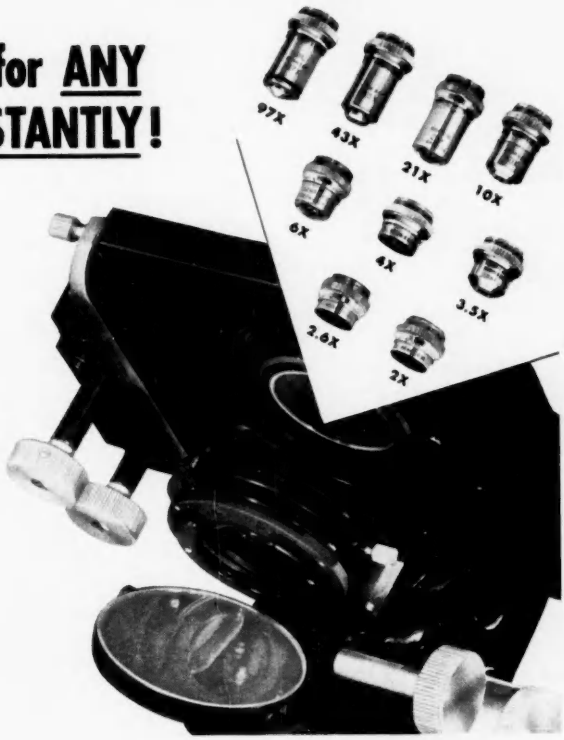

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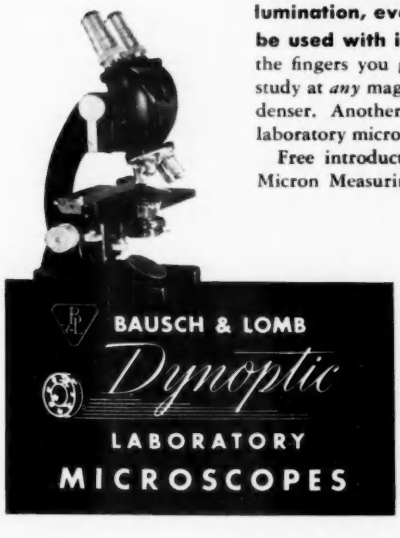

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AN INVESTIGATION INTO HYALURONIDASE AS A FACTOR IN THE MECHANISM OF TISSUE INVASION BY ENDAMOEBA HISTOLYTICA

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Differences in infectivity and/or invasion by *Endamoeba histolytica* have been ascribed to a variety of factors but the mechanism of these activities and the disease processes of amebiasis have not yet been satisfactorily explained.

The contribution of bacterial flora normally associated with *E. histolytica* to the mechanism of invasive activity has not been fully investigated. Duran Reynals (1939) described "spreading factors" for certain bacteria. Subsequent work has shown these "factors" to be hyaluronidase (McClean and Hale, 1941). Since many of the organisms which may produce hyaluronidase are normal inhabitants of the bowel, the hyaluronidase-hyaluronic acid enzyme system was investigated as one of the possible modes of invasion of tissue utilized by *E. histolytica*.

MATERIALS AND METHODS

The investigation of hyaluronidase-hyaluronic acid enzyme systems in *E. histolytica* was carried out in two parts: (I) *in vitro* studies performed in suitable culture media, and (II) *in vivo* studies using hamsters as host animals.

I. *In vitro studies.*

This series of investigations was in the nature of a survey to determine whether *E. histolytica* is capable of producing an extracellular hyaluronidase or an intracellular hyaluronidase-hyaluronic acid enzyme system.

The *in vitro* studies were divided into five phases, as follows:

(A). To test production of extracellular hyaluronidase under aerobic conditions.

(B). To test production of extracellular hyaluronidase under anaerobic conditions.

(C). To test possible inhibition of hyaluronidase activity by bacterial metabolites.

(D). To test the effect on hyaluronidase production of "seeding" ameba cultures with pure substrate.

(E). Assays for intracellular hyaluronidase.

[1] This work was sponsored by the Commission on Enteric Infections of the United States Armed Forces Epidemiology Board and supported by the Office of the Surgeon General, Department of the Army, Washington, D. C. Grateful acknowledgment is made to Mr. Peter J. Rennie for technical assistance and to the Allan Hancock Foundation of the University of Southern California for the use of certain of its facilities.

In these experiments one or more of the following strains of ameba and their associated flora were used: Faust-22, Denton, DKB,[2] and the Denton strain of ameba, grown in monobacterial culture with *Aerobacter aerogenes*.

To determine enzyme production, four test media were used: U.S.C. chemically defined medium (Hallman, Michaelson and DeLamater, 1950), Boeck and Drbohlav (1925) buffered whole egg medium, Cleveland-Collier (1930) liver medium overlaid with serum-Ringer's solution, and Balamuth's (1946) egg yolk infusion medium.

All experimental cultures were inoculated from laboratory stock cultures and incubated at 37.5°C. for 48 hours under both aerobic and anaerobic conditions.

After growth, test cultures were centrifuged at 2000 r.p.m. for 30 minutes and then Seitz-filtered. From 0.1 to 0.5 ml. aliquots of the centrifuged culture media were then assayed for hyaluronidase activity.

Enzyme activity was determined by the turbidimetric methods of Warren, Durso and Levin (1948), and Tolksdorf, McCready, McCullagh and Schwenk (1949). These methods were followed in absolute detail. All assays were run in triplicate and results were duplicated. As controls for these experiments the flora of each strain of ameba used was grown under the same conditions as the ameba. Hyaluronidase-producing strains of *Clostridium welchii* and *Micrococcus pyogenes, var. aureus*, were used as positive bacterial controls.

Bacterial and testicular hyaluronidases[3] were used to prepare standard curves for enzyme activity. Bacterial enzymes were prepared from stock cultures of *Cl. welchii* and *M. aureus*.

Potassium hyaluronate substrate used in these experiments was prepared in this laboratory from human umbilical cords, according to the procedure described by Tolksdorf, McCready, McCullagh and Schwenk (1949).

EXPERIMENTAL

(A). To test production of extracellular hyaluronidase under aerobic conditions.

In this phase of the *in vitro* studies all the strains of ameba and their associated flora, described above, were cultured on all of the test media.

Assays of cultures grown under aerobic conditions, showed no extracellular hyaluronidase production by any of the ameba strains or by their respective flora. *M. aureus* used as the positive control for this part of the *in vitro* investigations produced extracellular enzyme.

(B). To test production of extracellular hyaluronidase under anaerobic conditions.

The same strains of ameba and their associated flora and the same test media used in the study of hyaluronidase production under aerobic conditions were employed in this phase of the investigation; however cultures were grown under

[2] The Faust-22, Denton, and DKB strains were obtained through the courtesy of Dr. Fred Ryden.

[3] Testicular hyaluronidase was obtained from the Wyeth Institute of Applied Biochemistry, Philadelphia, through the courtesy of Dr. Joseph Seifter.

anaerobic conditions. Anaerobiasis was accomplished by using simultaneously the "candle" technique and sodium pyrogallate in anaerobic jars.

No enzyme activity was found in any of the culture media after growth except in that of *Cl. welchii*, the positive bacterial control.

(C). To test possible inhibition of hyaluronidase activity by bacterial metabolites.

1. *Demonstration of hyaluronidase inhibition.* Since a suitable substrate was present as indicated by hyaluronidase production by *Cl. welchii* and *M. aureus*, experiments were carried out to determine whether ameba or flora cultures were producing inhibitor substances. These experiments were conducted under aerobic conditions using the Boeck and Drbohlav whole egg medium with no added Bacto Rice powder. For this study a hyaluronidase-producing strain of *M. aureus* was grown in pure culture, in combination with *A. aerogenes*, and in culture with *A. aerogenes* and the Denton ameba. As shown in Table 1, enzyme activity was definitely inhibited in the culture of *A. aerogenes*, and in the culture of *A. aerogenes* with Denton ameba.

2. *Source of inhibition.* The nature of the inhibition was next studied to determine if the inhibition was due to specific substances produced by the ameba or its flora, or to substances inherently present in the medium. These experiments were done using the respective flora of the Denton and DKB strains of ameba and *A. aerogenes* as sources of inhibitor, and *M. aureus* and *Cl. welchii* cultures as enzyme sources. These cultures were grown on Boeck and Drbohlav medium overlaid with Stone's buffer without rice powder for 48 hours at 37.5°C. After incubation, cultures were centrifuged and Seitz-filtered, as described above. Aliquots of the ameba-flora cultures were then mixed with enzyme solutions in

TABLE 1

*AMEBAE STRAINS	NO. OF EXPERIMENTAL ANIMALS	NO. OF AMEBAE INJECTED PER ANIMAL	TIME OF SACRIFICE (DAYS FOLLOWING INJECTION)	HEPATITIS	HYALURONIDASE ACTIIVTY
Denton	8	20,000	5	None	None
	2	35,000	10	None	None
F-22 Ameba	4	20,000	5	None	None
	2	40,000	5	None	None
	2	27,000	10	None	None
DKB	2	20,000	5	Entire liver	None
	2	50,000	5	Entire liver	None
	2	2,000	10	None	None
NRS	2	10,500	5	None	None
	2	52,000	5	None	None
	2	25,000	10	None	None

* Unless indicated the amebae strains were cultured with their respective flora.

the proportions of one part of culture medium to one part of enzyme. These mixtures were then incubated in one group of experiments at 37.5°C. for 5 minutes, and in another group of experiments for 30 minutes at the same temperature. After incubation the mixed cultures were then assayed for activity. As controls, the floras of Denton ameba, DKB ameba and *A. aerogenes* were diluted 1:1 with 0.1 M acetate buffer (pH 6.0) and also with Stone's buffer (pH 7) and then were treated in the same way as the experimental flora cultures.

Results of these assays indicate that there is *no* apparent inhibitory factor produced by any of the floras. In addition, there was no inhibitory action due to Stone's buffer which was used to overlay the egg medium. The apparent inhibition of *M. aureus* enzyme product was found to be due solely to an overgrowth of *Aerobacter aerogenes* with a considerable decrease in the number of *M. aureus* organism in the culture and a consequent reduction in hyaluronidase production.

(D). To test effect on hyaluronidase production of "seeding" ameba cultures with pure substrate.

An attempt was made to induce the production of hyaluronidase by *E. histolytica* by adding potassium hyaluronate to the culture media (Rogers, 1945). Cultures used were the Denton strain of ameba with its usual flora, and the Denton strain of ameba grown monobacterially with *A. aerogenes*. *Cl. welchii* was used as the positive control. The culture medium used was whole egg, overlaid with Stone's buffer to which was added Bacto Rice powder.

To each tube was added 1 ml. of potassium hyaluronate solution (2.5 mg./ml.) which had been sterilized by Seitz-filtration. The tubes were incubated anaerobically for 48 hours at 37.5°C. Cultures were carried through five serial transplants before assays for hyaluronidase were made.

RESULTS

None of the test cultures showed any hyaluronidase production. *Cl. welchii*, the positive control, showed increased enzyme production after seeding of the culture medium.

(E). Assays for intracellular hyaluronidase.

Since *E. histolytica* is not capable of producing extracellular hyaluronidase, an experiment was performed to determine if hyaluronidase is contained in the ameba cell. The DKB and Denton strains of ameba and their respective floras were used in this assay. Cultures of these amebae were grown for 48 hours on Cleveland-Collier's medium to which was added Bacto Rice powder. After growth, the cultures were centrifuged for 15 minutes with 5 ml. of sterile serum-Ringer's. These washed cultures were then centrifuged and the supernate decanted. The residue containing amebae was then extracted with 0.1 M sodium chloride for 24 hours at 4°C. At the end of this time microscopic examination of the extracted residue revealed no living amebae. The solution containing the lysed ameba cells was then centrifuged for 15 minutes at 1500 r.p.m. This final

supernate was tested for hyaluronidase activity. Assays were made using the turbidimetric technics described previously. The respective flora of each strain of ameba was treated in the same manner and used as a control.

RESULTS

No intracellular hyaluronidase was found to be present in either of the ameba strains used or in their respective floras under the conditions of this experiment.

II. *In vivo studies.*

The possible mechanism by which *E. histolytica* is able to invade host organisms was next studied. This series of experiments was designed to determine if hyaluronidase has any role in the invasive mechanism of the ameba. Laboratory animals were infected with *E. histolytica*, the living amebae were then isolated from the infected animals and assayed for hyaluronidase production. One month old male and female golden hamsters weighing between 40–50 grams, were used as experimental hosts. Reinertson and Thompson's (1951) method of inducing experimental hepatitis was used.

Four stock strains of *E. histolytica*, Denton, DKB, F-22, and NRS were used for this study. During this investigation these amebae were cultured on Bacto Endamoeba medium, overlaid with 1:10 serum-Ringer's buffer to which finely milled Belgian rice starch had been added. The inocula were prepared by centrifuging 48-hour cultures of ameba, decanting the supernatant medium and suspending the sedimented amebae in sterile 1:10 serum-Ringer's solution. Infections were induced by direct injection of 0.05 ml. of inoculum containing 2,000 to 50,000 amebae, into the exposed livers of animals anesthetized with nembutal. A single inoculation was made into the ventral surface of the right lobe of the liver, so as to produce a visible bleb beneath the hepatic capsule. Seepage of the inoculum and bleeding were minimized by the use of a 27-gauge hypodermic needle.

It was not necessary to immunize the animals against the flora of any strain of ameba used. No deaths occurred due to bacteremia before the animals were sacrificed. Animals were arbitrarily sacrificed at 5 days and 10 days after inoculation.

Direct smears made of all lesions at the time of sacrifice were examined microscopically for amebae. When amebic abscessess resulted, cultures were made in Balamuth's and in Cleveland-Collier media. These cultures were incubated 48 hours at 37.5°C. and were assayed to determine the presence of extracellular hyaluronidase. Tolksdorf's method was used for these assays. All tests were made on Seitz-filtered cultures. The results of these experiments are found in Table 1.

Of the four strains of ameba used in the *in vivo* study, only the DKB strain produced typical amebic hepatitis. Assays of cultures of the ameba, isolated from the infected liver tissue showed no hyaluronidase activity.

A second series of experiments was then undertaken in order to determine whether bacterial flora or *E. histolytica* is the major etiologic agent in producing

hepatitis. In these experiments the hepatitis-producing DKB strain was used. The flora of this strain was mixed with the noninvasive Denton ameba strain, the natural flora of which had been suppressed by the use of antibiotics and this new system was used as one infecting inoculum. The DKB strain of ameba was cultured monobacterially with *Aerobacter aerogenes* and was used as the other infecting inoculum. These "criss-crossed" strains were cultured on Cleveland-Collier medium with added rice starch. Prior to inoculation into experimental animals the infecting inocula were prepared as previously described. Results of this experiment are found in Table 2.

A final series of experiments was performed to ascertain if hyaluronidase had any effect at all in producing amebic hepatitis. In this study pure testicular hyaluronidase, and bacterial hyaluronidase isolated from cultures of *Cl. welchii*, were mixed with cultures of the noninvasive F-22 ameba. These mixtures were then injected into the livers of hamsters. The animals were sacrificed after 5 days and examined for hepatic lesions and the presence of ameba. The results of these experiments are found in Table 3.

TABLE 2

AMEBA STRAINS	NO. OF EXPERIMENTAL ANIMALS	HYALURONIDASE ADDED (TRU)*	NO. OF AMEBAE INJECTED PER ANIMAL	TIME OF SACRIFICE (DAYS FOLLOWING INJECTION)	HEPATITIS	HYALURONIDASE ACTIVITY
F-22	2	30 (Testicular)	25,000	5	None	None
	2	25 (Testicular)	25,000	5	None	None
	2	60 (*Cl. welchii*)	25,000	5	None	None
Cl. welchii Culture medium	2	—	—	5	None	None

* TRU = *T*urbidity *R*educing *U*nit

TABLE 3

AMEBA STRAINS	NO. OF EXPERIMENTAL ANIMALS	NO. OF AMEBAE INJECTED PER ANIMAL	TIME OF SACRIFICE (DAYS FOLLOWING INJECTION)	HEPATITIS	HYALURONIDASE ACTIVITY
Denton amebae cultured with DKB flora	2	20,000	5	None	None
	2	33,000	5	None	None
	2	6,000	10	None	None
DKB flora	2	0.05 ml. of filtered undiluted medium	5	None	None
DKB (*Aerobacter aerogenes*)	2	3,000	5	Entire liver	None

DISCUSSION

I. *In vitro studies.*

Early experiments for hyaluronidase activity were done on culture media that had been centrifuged only. Results of hyaluronidase determinations on these culture supernates indicated some enzyme activity. Further investigation showed this activity to be due to viable bacteria, not removed by centrifugation alone, which were attacking the substrate. All subsequent cultures were subjected to Seitz-filtration after centrifugation and in none of these experiments was it possible to demonstrate any hyaluronidase activity except with the known producers of hyaluronidase, *Cl. welchii* and *M. aureus*. The significance of this finding points up the necessity of completely removing viable organisms from test solutions when assaying specifically for any extra-cellular enzyme or comparable substance.

Failure of *E. histolytica* to produce extracellular hyaluronidase *anaerobically* or *aerobically*, when *M. aureus* grown aerobically and *Cl. welchii* grown anaerobically produce the enzyme under the same cultural conditions, indicates a lack of synthetic processes for extracellular production of this enzyme by *E. histolytica*.

Since no inhibitor substance could be demonstrated by our technics, using either the 5-minute or 30-minute incubation times, we feel that this supports the concept that the hyaluronic acid-hyaluronidase enzyme system is not a product of *E. histolytica* metabolism.

Further failures of cultures to produce extracellular enzyme following seeding with pure potassium hyaluronate or intracellular enzyme under the conditions of our experiments, indicate a complete absence in *E. histolytica* of the synthetic mechanisms required for the production of hyaluronidase.

II. *In vivo studies.*

Our efforts to demonstrate an adaptive enzyme system both by seeding with substrate and by animal passage were stimulated by the work of Bradin (1951, 1953) who reported finding hyaluronidase in ameba cultures immediately after animal passage. We have no immediate explanation for the discrepancy in our results. It should be pointed out however, that Faust-22 was the only ameba common to both studies and these organisms were apparently maintained under different cultural conditions for a long period; that experimental technics vary somewhat; and that the evaluation of methods appear dissimilar. It would appear that a judicious appraisal of hyaluronidase production will require repetition using identical strains of ameba, and comparable methods and interpretations, before a satisfactory answer can be established.

Reinertson and co-workers (1952) criss-crossed bacterial flora of an invasive ameba and a non-invasive strain and failed to alter the infectivity for liver tissue of either. Our results are in agreement with theirs.

The failure of variations in flora to influence the invasiveness of the ameba strains which were used suggests that invasiveness may be an extracellular function of the ameba cell and that this may alter under conditions of culture and the strain of ameba being used.

There appears, however, to be a minimal concentration of invasive factor necessary for abscess production, because it was found that inocula containing fewer than 3,000 ameba of our DKB strain were ineffective in producing lesions. Inability of the Faust-22, Denton and NRS strains to produce hepatic lesions may therefore be based on quantitative factors rather than on complete loss of invasive function.

SUMMARY

Neither intra- nor extracellular hyaluronidase was produced by *E. histolytica in vitro* when amebae were cultured aerobically or anaerobically. No specific hyaluronidase inhibitor was produced by the flora of any strains of ameba cultured in this laboratory. Production of hyaluronidase was not induced when cultures of amebae were seeded with hyaluronate. Amebae, isolated from liver abscesses in hamsters, failed to produce hyaluronidase under the conditions used.

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IMMUNITY TO CERTAIN ARTHROPOD-BORNE VIRUSES AMONG INDIGENOUS RESIDENTS OF EGYPT

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The studies reported here were undertaken with a twofold purpose: to assist, principally by extending the surveys of immunity to West Nile virus, in inaugurating a collaborative program of virus investigations in Egypt, sponsored jointly by the laboratory of the U. S. Naval Medical Research Unit. No. 3, The Rockefeller Foundation, and the Ministry of Health of Egypt; and to acquire further information on the geographic range of certain viruses known or presumed to be arthropod-borne.

Melnick *et al.* (1951) proved beyond question that West Nile virus is present and even prevalent in Egypt. They isolated three strains of the virus from the blood of human beings and showed, by neutralization and complement-fixation tests, that in a number of localities immunity to this virus is acquired at a very early age. The persons from whom virus was isolated were not severely ill when the virus-yielding blood specimens were taken, and they exhibited no spectrum of symptoms or signs which could be regarded as a syndrome. Nevertheless, the West Nile virus is antigenically related to Japanese B, St. Louis (Smithburn, 1942; Casals, 1944) and Murray Valley encephalitis viruses (Pond *et al.*, 1952; Miles *et al.*, 1952; French, 1952)—agents which cause severe and sometimes fatal illness. Furthermore, it is known from the work of Southam and Moore (1953) that West Nile virus may also, on occasion, cause encephalitis in man. A study of the epidemiology of infection with West Nile virus was therefore given high priority when the joint program in Egypt was undertaken.

The first collection of blood specimens was made from outpatients at the Health Center at Sindbis village. Later an expedition travelled up the Nile to extend the survey to upper Egypt, and additional localities in the Nile delta were sampled, as shown on Map 1.

MATERIALS AND METHODS

Sera. Blood specimens were collected in sterile vacuum containers. Most of the very young children were bled from the jugular vein. Specimens were refrigerated promptly and continously until the serum could be removed. After separation from the clots, the sera were divided into two portions, one to be retained at the U. S. Naval Medical Research Unit No. 3 and one to be shipped to

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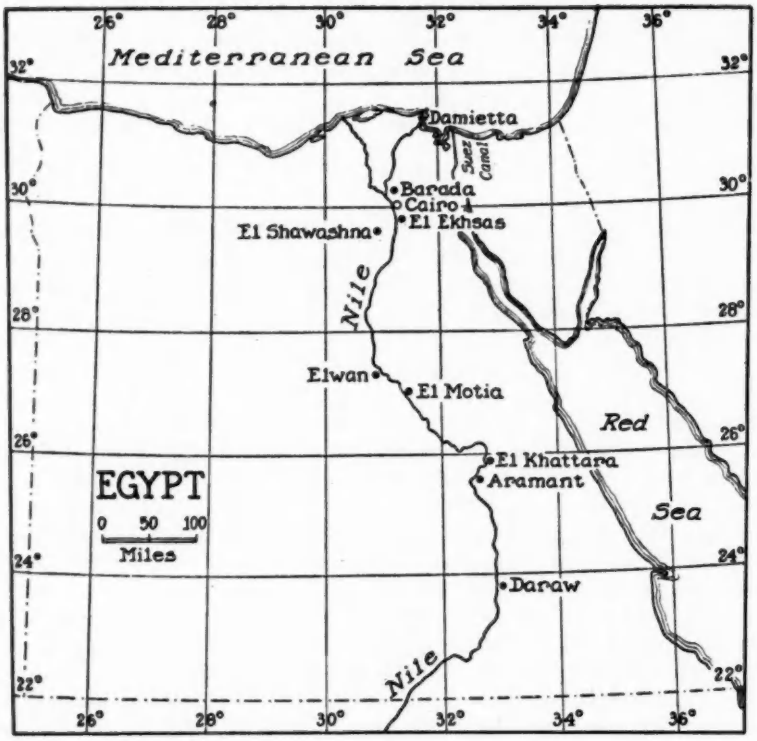

MAP 1

New York. As the volume of each sample received in New York was approximately 3 ml., the number of tests which could be done was limited. The separated sera were frozen and not permitted to thaw until required for testing. They were dispatched to New York by air under dry-ice refrigeration. In New York they were kept in a mechanical refrigerator at a temperature of about −20°C.

Viruses. The viruses used in these studies were all strains tested and adequately documented in earlier immunological studies (Smithburn, 1952). In some instances the same lots of virus were used, while in others one or two additional passages were made in the preparation of new lyophilized stocks. Previously assayed dry virus was used in all tests.

Neutralization tests. The methods employed in the tests were the same as those used in similar surveys made in East Africa (Smithburn, 1952a) and India (Smithburn *et al.*, in preparation). Sera were recorded as protective only when protection ratios of 4/5, 5/5, 5/6, or 6/6 were obtained in tests with 50 or more LD_{50} of virus. The intended dose of virus was 100 LD_{50}, and in most tests this dosage was exceeded.

RESULTS

This series of experiments comprised 50 test runs. The virus titers were variable in successive runs, and the virus dosage was therefore also variable. The range of virus dosage in the 50 tests is shown below.

DOSE (LD_{50})	NUMBER OF TESTS
under 50	9
50–99	12
100–199	8
200–299	10
300–499	8
500–999	2
1,000 and over	1

The mean, minimum, and maximum dosages for all tests with the ten different viruses are shown below.

VIRUS	NO. TESTS	DOSE (LD_{50})			NO. TESTS WITH DOSE UNDER 50 LD_{50}*
		Mean	Min.*	Max.	
West Nile	8	189	4	400	2
Ntaya	8	97	16	317	2
Japanese B	4	222(110)†	72	560	0
St. Louis	3	136	32	320	1
Uganda S	4	199	42	452	1
Zika	5	626(338)†	23	1,780	1
Semliki	5	190	125	282	0
Bunyamwera	5	158	58	400	0
Bwamba	5	100	32	263	2
Yellow fever	3	299	140	440	0

* Only negative results were accepted from tests with dosages under 50 LD_{50}. Sera giving inconclusive or protective results in such tests were re-examined. Only sera giving protection against 50 or more LD_{50} were recorded as positive.

† The figures in parentheses are the means obtained when single tests with an unusually high titer were omitted.

Table 1 summarizes all the neutralization tests without respect to residence of the donors, but with separate tabulations for children and adults.

Of the 140 sera tested against yellow fever virus, none was protective. None of the 231 sera tested against Bwamba fever virus gave evidence of neutralizing antibody. It seems probable that neither yellow fever nor Bwamba fever occurs in the localities from which the tested samples were derived. The localities concerned are listed in Table 2.

Of the 180 and 227 sera tested against Zika and Semliki Forest viruses respectively, one protective serum was found against each of these agents. The protective sera were both from adults. This result would indicate that these two

TABLE 1

Summary of neutralization tests against ten viruses with sera from indigenous residents of Egypt

VIRUS	CHILDREN*			ADULTS†			ALL AGES		
	No. tested	Protective		No. tested	Protective		No. tested	Protective	
		Number	Per cent		Number	Per cent		Number	Per cent
West Nile	142	78	54.9	189	144	76.2	331	222	67.1
Ntaya	140	29	20.7	187	85	45.5	327	114	34.9
Japanese B	56	1	1.8	87	11	12.6	143	12	8.4
St. Louis	59	0	0	88	2	2.3	147	2	1.4
Uganda S	80	0	0	98	4	4.0	178	4	2.2
Zika	80	0	0	100	1	1.0	180	1	0.5
Semliki	97	0	0	130	1	0.8	227	1	0.4
Bunyamwera	92	1	1.1	127	1	0.8	219	2	0.9
Bwamba	99	0	0	132	0	0	231	0	0
Yellow fever	63	0	0	77	0	0	140	0	0

* Under 15 years of age.

† 15 years of age and older.

agents are of little importance in the localities from which the tested samples were derived.

The sera of 1 of 92 children and 1 of 127 adults neutralized Bunyamwera virus. The immune child was a resident of El Motia (Table 2), and the immune adult lived in Elwan. No conclusions can be drawn from so few protective sera, but it is possible that Bunyamwera virus occasionally attacks human beings in Egypt.

None of 59 sera from children neutralized St. Louis encephalitis virus, but 2 of 88 sera from adults were protective. The two individuals from whom the protective sera were obtained resided in different localities. Both of the protective sera were also protective against West Nile, Japanese B, and Ntaya viruses. No conclusions can be drawn from this result.

None of 80 sera from children was protective against Uganda S virus, but 4 of 99 sera from adults neutralized this agent. The four immune donors, all of whom were male, resided in three different localities. In each instance a larger number of sera from adult females from the same localities were tested, with negative results. It is possible that Uganda S virus has been active in Egypt within the lifetime of persons now living. If so, it would appear that males may be attacked more frequently than females.

One of 56 sera from children and 11 of 87 from adults neutralized Japanese B virus. All 12 sera which were protective against this agent also neutralized West Nile and Ntaya viruses. All the sera tested against Japanese B virus were also tested against West Nile and Ntaya viruses, and many which were nonprotective against the former neutralized one or both of the latter. In view of the demonstrated fact (Melnick *et al.*, 1951) that infection with West Nile virus commonly

occurs very early in life in Egypt, it must be assumed that immune individuals are frequently re-exposed to infection during their lifetimes. It is possible that this gives rise to increased formation of group-specific antibody and accounts for the capacity of certain human sera—mainly from adults—to neutralize Japanese B virus. However, our knowledge at present does not permit us to come to a firm conclusion, and the investigator is obliged to remain open-minded as to whether or not Japanese B virus occurs in Egypt.

Ntaya virus was neutralized by 114 of 327 sera. The percentage rates for children and adults were 20.7 and 45.5 respectively. Many of the Ntaya-protective sera neutralized West Nile virus, but five were protective against Ntaya and negative against West Nile. The results indicate strongly that Ntaya virus occurs in Egypt and that the infection rate is roughly half that of West Nile virus. The frequent incidence of immunity to the two viruses in the same individual suggests that their epidemiological patterns may be similar—they may possibly have a common vector, or the antibodies to the viruses may be cross-reactive. The age and sex incidence and geographic distribution of immunity to Ntaya and West Nile viruses will be discussed in subsequent paragraphs.

The incidence of immunity to West Nile virus was much higher than for any other virus. Seventy-eight of 142 sera from children (54.9 per cent) and 144 of 189 from adults (76.2 per cent) neutralized this agent. Many sera neutralized West Nile virus and were nonprotective for all others against which they were tested. The results are in general agreement with those of Melnick *et al.* (1951) and have been confirmed and extended by Taylor *et al.* (unpublished). Additional information on the results obtained with West Nile virus is discussed in the following paragraphs.

Geographic range and age and sex incidence of infection with West Nile and Ntaya viruses. The results of the survey are summarized by locality of residence and age and sex of donors in Table 2.

The data pertaining to age, sex and residence of donors require no further comment for any of the viruses except West Nile and Ntaya. The quantity of each serum sample received in New York did not permit all specimens to be tested against ten viruses. However, all were tested against West Nile virus, and all but four were tested against Ntaya. Five sera which neutralized Ntaya virus failed to neutralize any of the other agents against which they were tested. These five Ntaya protectives were discovered in three different test runs in which the virus dosage was 80, 80, and 317 LD_{50} respectively, with two protective sera in each of the 80 LD_{50} runs and one in the 317 LD_{50} run. The latter serum gave negative results not only against West Nile, but also against St. Louis and Japanese B viruses. The other four were tested against Bwamba fever, Semliki Forest, Bunyamwera, Uganda S, Zika, and yellow fever viruses in addition to West Nile, but they neutralized only Ntaya. All individuals from whom these samples were taken were females. Two were children, 7 and 12 years of age; three were adults, two aged 40 and one aged 35. They represent the localities of Daraw, Aramant, and El Khattara. Males immune to Ntaya virus were found in each of these localities, but their sera also neutralized West Nile virus.

TABLE 2

Results of neutralization tests against ten different viruses with sera from indigenous residents of Egypt, showing age, sex, and residence of donors*

RESIDENCE OF DONORS	AGE GROUP	WEST NILE		NTAYA		JAPANESE B		ST. LOUIS		UGANDA S		ZIKA		SEMLIKI		BUNYAM-WERA		BWAMBA		YELLOW FEVER	
		M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Damietta	children	0/6	0/7	0/6	0/7	0/6	0/7	0/6	0/7					0/6	0/7	0/6	0/7	0/6	0/7		
	adults	0/7	1/8	0/7	0/8	0/7	0/8	0/7	0/8					0/7	0/8	0/7	0/8	0/7	0/8		
Barada	children	4/7	5/7	1/7	1/7					0/7	0/7	0/7	0/7	0/6	0/6	0/4	0/5	0/7	0/7		
	adults	2/4	12/12	0/4	2/11					1/3	0/11	0/4	0/12	0/3	0/11	0/2	0/9	0/4	0/12		
Cairo†	children	2/8	1/2	1/8	0/2	0/8	0/2	0/8	0/2					0/5	0/1	0/5	0/1	0/5	0/1		
	adults	13/28	4/4	9/28	2/4	3/28	0/4	1/28	0/4					0/17		0/17		0/17			
El Ekhsas	children	8/10	5/5	3/9	0/4					0/10	0/5	0/10	0/5	0/10	0/5	0/9	0/4	0/10	0/5	0/8	0/4
	adults	6/6	9/10	5/6	2/9					1/6	0/10	0/6	0/10	0/6	0/10	0/6	0/10	0/6	0/10	0/6	0/7
El Khattara	children	0/6	4/7	0/6	1/7	0/6	0/7	0/6	0/7												
	adults	8/10	5/9	7/10	2/9	3/10	0/9	1/10	0/9												
El Shawashna	children	1/6	3/7	0/6	0/7	0/6	0/7	0/6	0/7												
	adults	11/11	1/3	6/11	0/3	3/11	0/3	0/11	0/3												
El Motia	children	6/10	5/5	2/10	1/5					0/10	0/5	0/10	0/5	0/10	0/5	1/10	0/5	0/10	0/5	0/10	0/5
	adults	7/7	8/8	5/7	3/8					0/7	0/8	0/7	0/8	0/7	0/8	0/7,	0/8	0/7	0/8	0/6	0/8
Elwan	children	2/3	7/10	1/3	2/10					0/3	0/10	0/3	0/10	0/3	0/10	0/3	0/10	0/3	0/10	0/3	0/10
	adults	4/4	13/13	3/4	2/13					2/4	0/13	1/4	0/13	1/4	0/13	0/4	1/13	0/4	0/13	0/4	0/13
Aramant	children	1/3	5/8	0/3	5/8					0/3	0/8	0/3	0/8	0/3	0/8	0/3	0/8	0/3	0/8	0/3	0/8
	adults	10/11	8/9	9/11	7/9					0/11	0/9	0/11	0/9	0/11	0/9	0/11	0/9	0/11	0/9	0/10	0/9

Daraw	children	7/12	12/13	4/12	7/13	0/4	1/3	0/5	0/5	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
	adults	10/11	12/14	9/11	12/14	1/3	1/4	0/5	0/3	0/6	0/10	0/6	0/10	0/6	0/10	0/6	0/10	0/6	0/10	0/5	0/9
Totals	children	31/71	47/71	12/70	17/70	0/30	1/26	0/31	0/28	0/39	0/41	0/39	0/41	0/49	0/48	1/46	0/46	0/50	0/49	0/30	0/33
	adults	71/99	73/90	53/99	32/88	10/59	1/28	2/61	0/27	4/37	0/61	1/38	0/62	1/61	0/69	0/60	1/67	0/62	0/70	0/31	0/46

* For all ratios given, numerator represents number of sera protective and denominator number of sera tested.

† Includes a special collection from 11 adult males employed at the abattoir, Heliopolis, in whom the results were similar to those for Cairo adults.

The high incidence of immunity to West Nile virus early in life found by Melnick *et al.* (1951) is confirmed by the results reported here. Of donors under 15 years of age 54.9 per cent had protective sera. The rate in female children (66.2 per cent) was higher than in male children. It was also higher in adult females (81.1 per cent) than in adult males (71.7 per cent), suggesting that infection is contracted in the environs of the home. Also, females appear to be either more frequently exposed or more susceptible to infection than males.

The rates for Ntaya virus, though lower than for West Nile, nevertheless showed that immunity is commonly acquired early in life. Of all sera from children 20.7 per cent were protective. In males the rate was 17.1 per cent, and in females it was 24.3 per cent. In adult males, however, the rate (53.5 per cent) was higher than in females (36.4 per cent). Thus, the higher incidence of infection in female children, if genuine, apparently is not carried over into adult life.

The localities of Damietta and El Shawashna, each represented by 13 specimens from children, showed no immunity to Ntaya virus in this age group. All the adults whose sera were protective at El Shawashna were males; it is possible that they acquired their infection elsewhere. More tests on specimens from both children and adults at El Shawashna would be required before a conclusion as to the occurrence of Ntaya virus infection there could be drawn. However, the 15 adults tested at Damietta were all nonimmune to Ntaya virus; it therefore seems not unreasonable to conclude that infection with this agent does not occur or at least is uncommon there.

Damietta was the only locality in which no children immune to West Nile were found; only 1 of 15 adults was immune. Thus the incidence of infection with this virus at Damietta is very low, and the difference between rates for this locality and all others is highly significant. This fact has been confirmed in separate studies made by one of the authors (RMT) and an attempt has already been made to find factors which may explain the difference. These investigations will be reported at a later date.

Elsewhere, in all localities sampled, immunity to West Nile virus was quite common in both adults and children. The highest rates were at El Ekhsas, Daraw, El Motia, and Elwan, where the incidence in children was 86, 76, 73, and 73 per cent respectively. Thus, infection with this agent is common not only in the environs of Cairo, but in localities along the Nile in upper Egypt.

DISCUSSION

In surveys of antiviral immunity involving the testing of individual sera against a multiplicity of viruses, the occurrence of plural protection poses a problem to which present knowledge does not offer a ready solution. When an individual serum neutralizes two or three viruses, the question at once arises as to whether the individual has actually been infected with all the corresponding viruses or whether some of the reactions are due to group-specific antibody. From studies already completed (1–4, 6–10, 12) it is known that antigenic interrelationships exist between all agents included in this study except Semliki

Forest, Bunyamwera, and Bwamba fever viruses. However, these cross-reactions have been elicited for the most part with laboratory-produced antisera, and there is little information on the frequency with which they occur in human beings convalescent from natural infections. In immunized or hyperimmunized animals the heterologous antibody response varies from one individual to another and is not related to the quantitative production of homologous antibody (Smithburn, 1952). Fortunately, the cross-reactions involving some of the viruses, notably yellow fever, Uganda S, and Zika, appear to be unilateral and/or of minor order and are unlikely to give rise to confusion. However, the antigenic relationships involving West Nile, St. Louis, and Japanese B (also Murray Valley) viruses are more intimate, more likely to be reciprocally reactive, and quantitatively stronger. Therefore, unless the virus can actually be isolated or sera found which neutralize that agent alone, it may be unjustified to conclude that "significant" neutralization of a certain virus by sera from residents of a certain area is indicative of the presence of that virus in the area. For these reasons we hesitate to conclude that the results reported here are firm evidence that Japanese B virus is present in Egypt.

In the case of Ntaya virus, the evidence for the occurrence of the agent in Egypt is substantial. Not only did a high percentage of individuals, including children, exhibit protective antibody, but some of these were protective against this agent alone. The same is true of West Nile virus, and for this agent there is the additional evidence of repeated isolations in Egypt (Melnick *et al.*, 1951; Taylor *et al.*, 1953). However, since Ntaya and West Nile viruses are known to be antigenically related (Smithburn, 1952; Smithburn, Casals, unpublished), it is possible that some of the many Egyptian residents whose sera neutralized both of these agents may actually have suffered infection with only one of them. If so, quantitative tests might settle the point. Experiments of this nature are currently in progress in these laboratories.

SUMMARY

Sera from indigenous residents of ten widely scattered localities in Egypt were tested for capacity to neutralize ten different viruses, each known or believed to be arthropod-borne. The following results of significance were obtained.

1. Immunity to West Nile virus is very prevalent in all localities sampled except Damietta. Immunity is frequently acquired very early in life. Many sera neutralized this virus and no other.

2. Protective antibody against Ntaya virus was demonstrated in 34.9 per cent of all sera tested. Protective antibody against this agent was commonly associated with capacity to neutralize one or more other viruses, particularly West Nile. A few sera neutralized Ntaya virus alone. The only locality showing no immunes was Damietta.

3. Twelve of 143 sera neutralized Japanese B virus, but since the same sera also neutralized West Nile and Ntaya viruses, this result cannot be interpreted at present.

4. A few sera neutralized Uganda S, St. Louis encephalitis, Bunyamwera, Zika, and Semliki Forest viruses, but the results do not indicate that these agents are medically important in any of the localities concerned.

5. None of the sera tested against Bwamba fever or yellow fever viruses neutralized these agents.

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INDUCED VIRUS INFECTIONS IN MAN BY THE EGYPT ISOLATES OF WEST NILE VIRUS[1, 2]

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The study of pedigreed infections in man in which the character of the inoculum and the time of inoculation are known, and the subsequent behavior of the pathogen can be followed, is only rarely possible. The observations that certain viruses have an antineoplastic effect in experimental animals has led to the deliberate induction of certain virus infections in patients with advanced cancer as an experimental therapeutic method. As a by-product of these studies, a detailed analysis of these virus infections and of factors influencing them has been possible. The literature concerning oncolytic effects of viruses in experimental animals has been reviewed through 1951 (Southam and Moore, 1952). More recent papers report oncolytic effects of virus against myxoma of rabbits (Ginder and Friedewald, 1952), Ehrlich ascites tumor of mice (Koprowska *et al.* 1953; Love *et al.* 1953), and mouse leukemia (Southam and Epstein, 1953).

In a previous paper (Southam and Moore, 1951) the clinical course of induced infections in man by West Nile, Ilheus, and Bunyamwera viruses was presented. Since that report, there have become available three new isolates of West Nile virus known as Egypt 101, Egypt 21 and Egypt 19 (Melnick *et al.*, 1951). These three viruses were isolated from the sera of persons with little or no recognized illness, and were of interest to the present authors because it was felt that their recent isolation from a human source might make them more infectious for man than the previous West Nile isolate which had been through 140 or more mouse brain passages before clinical trial as an antineoplastic agent. This proved to be true, and the subsequent observation of occasional regression of neoplastic disease during the course of this virus infection prompted the extended use of this virus as an experimental therapeutic agent in patients with advanced cancer. The present report concerns the clinical course of the induced virus disease, and the behavior of the Egypt viruses in these patients. Several recent isolations of West Nile virus from blood of symptomatic patients in Egypt (Taylor, pers. commun.) and in Israel (Bernkopf; Goldblum 1953), and the recent demonstration of specific antibodies in the sera of natives in India (Smithburn, 1953) suggest that this virus may be of more than academic interest.

The effects of the virus infection upon the patient's neoplastic disease will be

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mentioned only briefly in this paper. The evidence for anti-neoplastic effects of this virus in man has been discussed in an earlier publication (Southam and Moore, 1952). Detailed studies of the serology and pathology associated with this infection will be published elsewhere (Southam and Moore, in prep.; Newman and Southam, 1954).

METHODS

All patients had neoplastic disease of such a type or extent as to be nonresponsive to surgery, x-ray therapy, or established methods of chemotherapy. All patients were volunteers, and were informed of the experimental nature and the infectious nature of the virus inoculation. A wide spectrum of neoplastic diagnoses was represented (Table 1). General clinical status of the patients varied widely. Life expectancies of the patients ranged from hours to two or three years, but the majority were in terminal condition, as reflected by the fact (Table 1) that 27 per cent of the patients died due to their neoplastic disease within three weeks of admission.

Patients were hospitalized in a screened isolation unit and were cared for by specially trained medical and nursing personnel. Infectious disease precautions were maintained for three weeks following each virus inoculation, with emphasis on blood and needle precautions.

Heparinized blood was drawn daily for virus isolation, and clotted blood was drawn weekly for antibody studies. Tumors were biopsied for virus isolation and histology at intervals of four or five days whenever feasible. Prior to inoculation each patient was thoroughly studied for general medical evaluation, and particularly for objective evaluation of the patient's neoplastic disease. Such evaluation studies were repeated at the end of the three weeks isolation period, and as frequently as practicable thereafter.

The three Egypt isolates of West Nile virus were obtained as the third passage in mouse brain or in chick embryos. Stocks for clinical use were made from this original material by one further passage in the same host. All patients were inoculated with this fourth passage material (egg or mouse), or with first mouse-brain passage material following isolation from a previous patient in this series, or with infected human tumor tissue. All inoculations were intramuscular, except two which were intraperitoneal. The inoculum was a 10 per cent or 20 per cent crude tissue emulsion, and varied from 2 to 8 cc. of a 10 per cent tissue suspension, or equivalent of 20 per cent suspension. Virus preparations were stored as whole tissues or saline suspensions until immediately prior to inoculation in a solid-CO_2 box, in rubber stoppered tubes or sealed ampoules.

Virus was detected by intracerebral inoculation of 12 to 15 gm. white mice. Fluids were tested undiluted. Tissues and feces were tested as 10 per cent suspensions. Materials contaminated with bacteria were filtered through a Swinney filter prior to inoculation. Death of mice between five and ten days after inoculation and preceded by paralysis was considered indicative of virus. When the pattern of death of test mice was atypical, brains were examined for pyogenic infection, a second passage was made from the suspected mouse brains, filtration

was used to exclude possible bacteria, and, when necessary, neutralization studies were performed with known antisera. Titrations were recorded as the LD_{50} dilution, as computed by the method of Reed and Muench (1938).

The charted antibody titers were obtained by standard complement fixation and neutralization techniques. Neutralization techniques utilized undiluted serum and serial dilutions of virus. Details of these techniques will be presented in a subsequent publication.

The following explanations apply to all charts and tables. The 24 hours following virus inoculation is called day 0. From 24 to 48 hours after inoculation is day 1, etc. *Persistence* of viremia is indicated as the latest day on which virus was demonstrated in blood. In many patients the *duration* of viremia was shorter than this indicated number of days because virus was not detectable in blood until day 2 or later. A few patients died while viremia still persisted. In order to include such data in averages it was arbitrarily assumed that viremia persisted one day longer than demonstrated in these few patients. Clinical illness attributable to the virus infection is defined as follows: 0, no reaction except fever of not over 1° F.; +, fever of 1° to 3° F. only; ++, fever over 3°F, but with no neurologic signs or symptoms; +++, possible encephalitis with neurologic effects persisting not more than 48 hours; ++++, definite and more persistent encephalitis. Severity of fever was determined by comparison with the pre-virus base line, which in these patients was often slightly above "normal." Temperature curves are recorded in terms of daily maxima. Oncotropism, the localization of virus in tumor tissue, is tabulated as "selective" if virus was present in higher titer in tumor than in normal tissues obtained and tested simultaneously (difference of one log or greater), or if virus was present in tumor 48 hours or longer after blood became virus-free. Oncotropism is tabulated as "yes"' if virus was present in tumor tissue but the criteria of selective localization were not satisfied, and as "no" if virus was not demonstrable in tumor tissue when it was present elsewhere. Tumor regression ("oncolysis") is tabulated as "yes" when definite objective regression of tumor masses was demonstrated by direct measurement or by x-ray at a time consistent with a virus effect; and as "?"when there was suggestive or very transient tumor regression by the above criteria, or (in two patients) as judged by serial Papanicolaou studies of Class V effusions (Koprowska, 1953). The patients' subjective responses are eliminated from these evaluations, although statements of subjective improvement were frequent. The symbol "x" in tables indicates data is lacking.

RESULTS

Egypt 101 isolate of West Nile virus

Ninety-five patients were inoculated with the Egypt 101 isolate of West Nile virus. Three had previously been inoculated with other isolates of West Nile virus. Since there was no evidence of propagation of Egypt 101 virus, these three patients will not be discussed further in this paper. Two patients were inoculated intraperitoneally and will be considered separately. Six died less than six days after inoculation. These will not be considered further except to note that their

death was not causally related to the virus infection. Thus, 84 patients lived long enough after intramuscular inoculation of Egypt 101 virus for determination of the persistence and distribution of the virus (Table 1). Six of the 14 patients who died from 6 to 13 days following virus inoculation could not be evaluated concerning clinical course of the infection because pre-existing disease obscured any clinical evidences of the virus infection which may have developed. Evaluation of anticancer effects was not attempted for patients who died sooner than 14 days following virus inoculation, with two exceptions, because tumor regression (oncolysis) did not become apparent until three weeks or longer after inoculation. The two exceptions died on day 12 but had evidence of tumor regression. One of these patients (M. M., 54, M) is tabulated as definite tumor regression. He had rectal adenocarcinoma and has been discussed in a previous publication (Southam and Moore, 1952). The other patient (R. D., 60, M) had lymphosarcoma and is tabulated as "?" regression. He exhibited suggestive diminution of splenomegaly and striking decrease in neoplastic cells in pleural and peritoneal effusions. Data concerning localization of virus in tumor tissue (oncotropism) is available only for the 56 patients in whom biopsy of tumor was possible, or who died and were autopsied while virus was still present in their tissues.

Clinical course. In 89 per cent of the 78 patients available for evaluation, Egypt 101 infection was characterized by an absence of any clinical signs or symptoms other than fever, and in 27 per cent fever never exceeded 1°F. (Table 1). Fever usually started 24 hours after virus inoculation and persisted during the period of viremia. A secondary febrile period occurred in a few patients during the third week after inoculation. Figures 7 to 18 present fever curves and viremia data of representative patients in each of the five categories of clinical severity.

Nine (11 per cent) of these patients showed definite or suggestive clinical signs of diffuse encephalitis. When encephalitic signs or symptoms occurred they usually started during defervescence of the initial or secondary febrile period. The evidences of encephalitis were depressed sensorium, ranging from drowsiness to near-coma; involuntary twitching of hands, and occasionally of face and legs; and irregular variation of deep tendon reflexes, usually hyperactivity followed by diminished activity. Cogwheel rigidity was usually observed. Paralysis or paresis occurred only once and recovery was complete (patient J. E., 64 M). Pyramidal tract signs were observed only in the one patient with paralysis. Cranial nerves and sensory perception were never affected. Headache was a complaint in only two patients and appeared to be of only moderate severity. Slight papilledema appeared in these same two patients. Meningitic signs did not occur. (Many patients were receiving analgesic agents because of pain arising from their cancer, so headache of mild severity might have been masked.) Case histories of two patients representative of the definite or suggestive encephalitic reactions are appended.

Spinal fluid findings are summarized in Table 2. In patients without encephalitis spinal fluid abnormalities due to virus were not observed. Among eight patients with +++ or ++++ encephalitis there was little or no abnormality of pressure or cells. (The patient S. B., 45, M, had extreme leukemic leukocytosis

TABLE 1

Egypt 101 virus, summary of 84 patients

Figures indicate number of patients in each category

CANCER DIAGNOSIS	NO. PTS.	DEATH (Days after virus)			VIREMIA (Latest day)					ILLNESS DUE TO VIRUS					ONCOTROPISM			ONCOLYSIS		
Type and Origin		6-13	14-21	>21	0	1-2	3-5	6-12	>12	0	+	++	+++	++++	No	Yes	Select	No	?	Yes
Epidermoid:																				
Head & Neck	7	—	1	6	—	3	1	2	1	1	4	1	—	—	—	3	1	6	1	—
Gyn	3	—	—	3	—	—	3	—	—	1	2	—	—	—	1	—	2	3	—	—
Broncho	2	—	1	1	—	—	1	1	—	1	—	—	—	—	1	—	1	1	—	—
Other	4	—	1	3	—	2	—	2	—	1	2	—	—	1	1	2	1	4	—	—
Adenocarcinoma:																				
Colon	27	2	1	24	—	—	4	23	—	4	16	5	1	—	—	14	3	19	2	3
G.I.	8	—	—	8	1	1	1	5	—	5	1	1	—	1	—	—	2	5	3	—
Breast	5	1	1	3	—	—	—	5	—	1	2	2	—	—	—	1	1	4	—	—
Other	5	1	1	3	—	1	1	3	—	3	—	1	—	1	1	3	—	4	—	—
Sarcoma:																				
R.E.S.	8	3	2	3	1	—	—	1	6	—	3	—	2	3	—	5	1	2	2	3
Melanoma	7	4	—	3	—	—	—	6	1	2	1	2	—	—	—	6	—	5	1	—
C.N.S.	4	1	1	2	2	—	1	1	—	—	3	—	—	—	—	1	1	2	—	—
Other	4	2	—	2	—	—	—	4	—	2	2	—	—	—	1	1	1	1	1	—
Sub totals		14	9	61	4	7	12	53	8	21	36	12	3	6	6	36	14	56	10	6
Totals	84	84			84					78					56			72		

TABLE 2

Spinal fluid findings in 14 patients during Egypt 101 and Egypt 21 virus infections

PATIENT, AGE & SEX	DEGREE OF CLINICAL ILLNESS	DAY OF L.P.	INITIAL PRESSURE	CELLS	PROTEIN	SUGAR	VIRUS IN		DAY OF DEATH	ENCEPHALITIS AT AUTOPSY
							CSF	Blood		
Egypt 101 Virus										
S. B., 45, M	++++	10	200	129	116.9	86	0	0	13	Yes
B. C., 31, F	++++	14	160	40	30.6	103	0	+		
		17	260	15	31.3	83	0	0	18	x
A.D., 42, M	++++	12	120	2	40.4	67	+	0		No
		17	x	x	36.7	69	0	0	28	(cord only)
R. M., 43, F	++++	12	260	2	11.1	94	0	+		
		18	80	0	23.7	68	0	+		
		24	x	0	63.6	49	0	0	54	No
J. E., 64, M	++++	7	180	40	42.1	89	+	+	Living at	
		14	120	10	92.4	90	0	0	12 months	
W. L., 66, M*	++++	0	70	bloody	133.0*	100	x	x		
		8	x	bloody	66.0	100	+	+	17	x
R. D., 60, M	+++	2	194	2	14.2	69	x	+		
		5	160	1	20.5	52	0	+		
		10	200	2	x	x	0	+	12	No
L. R., 68, M	+++	15	80	17	63.8	51	0	0	62	No
A. G., 59, M	++	5	70	x	46.2	67	0	+	Living at	
		6	65	0	27.1	77	0	+	4 months	
		12	132	13	x	62	0	0		
J. K., 38, M	+	1	280	x	124.2*	85	0	0		
		6	600	x	126.0	78	0	0		
		8	300	x	92.9	67	0	0		
		13	140	x	133.0	80	0	0	15	x
J. S., 27, M	+	2	290	3	19.7	53	0	+	Living at	
		5	200	6	16.9	54	0	+	7 months	

Egypt 21 Virus

P. K., 73, M	+++	13	220	20	33.5	108	0	0		
		16	180	18	42.9	111	0	0	20	x
P. T., 47, M	+	13	180	x	38.0	73	0	0	Living at 6 months	
C. L., 62, M*	+	3	80	1	59.6	54	0	+	35	x

* Elevated spinal fluid pressure and protein in patient J. K., 38, M were due to brain tumor.
Patient W. L., 66, M had tumor involvement of cord causing paraplegia.

so the significance of cells in his spinal fluid is not known.) Protein content was above normal levels in five patients. Of the three patients having clinical signs of encephalitis but without elevation of spinal fluid protein, two showed no evidence of central nervous system pathology at autopsy (only cord examined from patient A. D., 42, M) and autopsy permission was refused on the third. On the basis of clinical and histopathalogic evidence it is concluded that the virus infection did not cause the death of any patient, but the possibility cannot be excluded that encephalitis was a contributing factor in the death of patient S. B., 45, M.

The advanced neoplastic disease in these patients often made precise determination of the cause of various symptoms difficult. Therefore, a categorical discussion of signs and symptoms seems in order. Many patients in this series had anorexia and nausea, and some had vomiting, due to intra-abdominal cancer or due to uremia. These patients occasionally attributed an apparent increase in symptoms to the virus inoculation, but to the physician these complaints seemed no different from those which preceded virus inoculation. Patients without preceding gastro-intestinal disturbances had none following virus inoculations. Diarrhea was never attributable to virus, nor was jaundice. More than half of these patients had liver function studies before and three weeks after virus inoculation, and in no case did any changes occur except those to be expected from proved metastatic involvement of the liver. No respiratory signs or symptoms occurred. No cardiac pathology attributable to virus was detectable clinically or in four patients on whom electrocardiograms were taken three times weekly during the period of virus study. The development of cardiac decompensation in one patient (L. R., 68, M) during the febrile period of his virus infection was adequately explained on the basis of arteriosclerotic heart disease and pulmonary hypertension due to metastatic disease in the lungs and bronchopneumonia. Two patients developed vesicular skin lesions on the thorax during the second week after virus inoculation. They healed in a few days without scarring. An etiologic relationship to the virus seems doubtful, but no other cause was found. The vesicular fluid did not contain virus. No other skin lesions, specifically no macular or petechial rashes, occurred with the exception of two patients who showed typical drug rash which responded to treatment with antihistaminics and withdrawal of the offending drug. Leukocyte counts and differentials were performed three times weekly on a series of 29 consecutive patients. Analysis of these data showed no consistent change in the total leukocyte count or differential. Specifically, there was no relative or absolute lymphopenia.

Virus recoveries. Persistence of viremia is summarized in Table 1. Blood contained virus at 24 hours or later after virus inoculation in all except 4 (5 per cent) of the 84 patients. In an additional seven patients, making a total of 13 per cent, virus was not recovered longer than 48 hours following virus inoculation. In accordance with our previous interpretation, such patients are considered as "no take," since it is possible that virus persisting for only 48 hours might be residual from inoculum rather than newly propagated virus. The remaining 87 per cent of patients had virus infection as judged by viremia on day 3 or later. Furthermore,

73 per cent had viremia 6 days or more after inoculation, and 10 per cent had viremia longer than 12 days. The large percentage of patients with prolonged viremia stands in marked contrast to previous results with the original strain of West Nile virus (high mouse passage) with which infection occurred in only five of 21 patients and viremia persisted for 6 days in only two (Southam and Moore, 1951).

In many patients, virus was not detected in the blood at 24 hours after inoculation but was present at 48 hours and persisted thereafter as indicated above. In two patients the onset of viremia occurred even later. One of these (R. F., 36, F) had detectable virus in her blood for the first time on day 4 after which it persisted through day 7. The other (J. M., 61, M, Fig. 12) first had viremia on day 5, following which it persisted through day 13 and was not tested for thereafter because it had been presumed on the basis of earlier evidence that infection had not been established. In this patient the inoculum was of unusually low titer (10^{-2}) and this may account for the delayed viremia, however, the inoculum for the first patient had a titer of $10^{-8.0}$ so this factor obviously did not operate in her case.

Virus titrations were performed on bloods of 8 consecutive patients (Fig. 1). Titers increased rapidly to reach maxima of 10^{-2} to greater than 10^{-5} at three or four days following inoculation, and thereafter diminished somewhat less rapidly to disappear in most cases between the 9th and 14th days. The patient E. C., 35, F (Fig. 10 and case history) had a greatly prolonged viremia (28 days), perhaps due to depression of reticulo-endothelial function (see Discussion).

Virus was not recovered from throat washings, stools, or urines (Fig. 2) even during the period of viremia. Virus was recovered from cerebrospinal fluid of

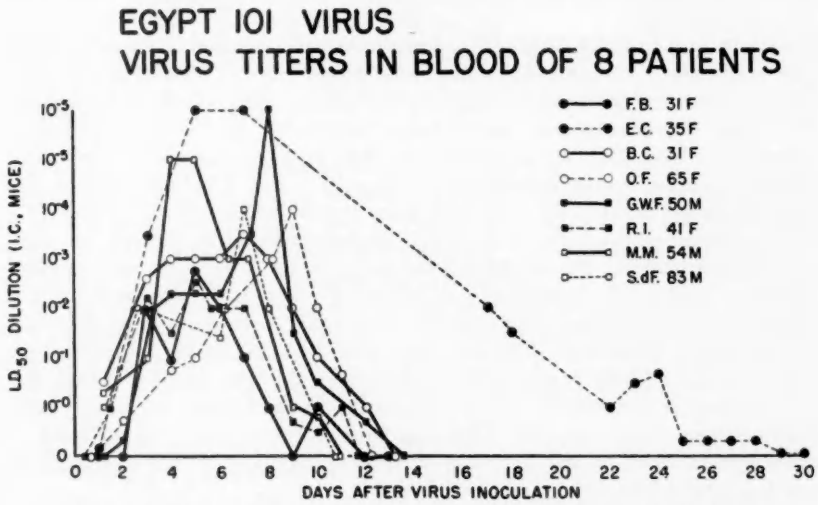

FIG. 1. Titers of Egypt 101 virus in blood of 8 randomly selected patients

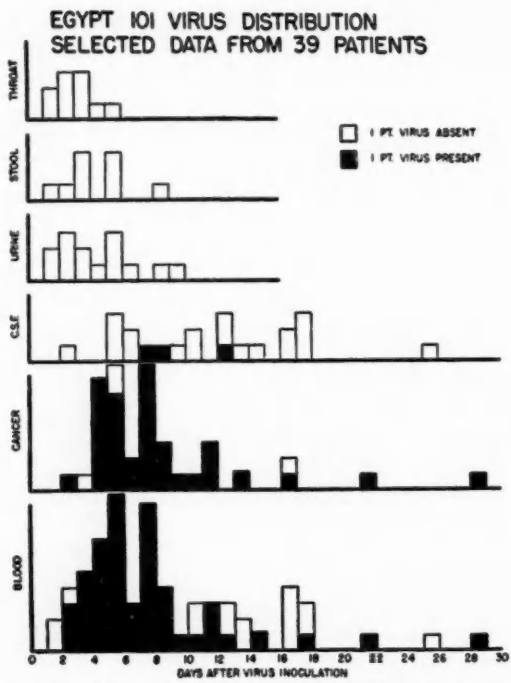

FIG. 2. Distribution of Egypt 101 virus in throat washings, feces, urine, cerebro-spinal fluid, and tumor tissue of 39 patients; and in blood collected simultaneously.

three patients. In two patients this occurred during the period of viremia, but in the other patient it occurred five days after blood was virus free. Each of these three patients had definite encephalitis or encephalomyelitis (see Case Abstracts). The virus recovered from spinal fluid of patient A. D., 42, M was identified as Egypt 101 (West Nile) by neutralization tests. In five other patients who had clinical evidence of encephalitis due to Egypt 101, and three who did not, the spinal fluid did not contain virus even when tested during viremia.

Tissue localization of virus. Virus was frequently demonstrated in tumor tissue, and in some patients was *selectively* localized in tumor. This was clearly demonstrated in some of the autopsied cases indicated in Fig. 3. Detailed presentation of data concerning oncotropism of Egypt 101 virus is, however, beyond the scope of this paper. Some such data has been published previously (Southam and Moore, 1952) and publication of further data on this phenomenon is planned. Among normal tissues (Fig. 3) virus was most frequently isolated from lymph nodes, spleen, lung, and liver. This suggests that virus may be distributed to those viscera which have a high concentration of reticulo-endothelial cells. Virus was less often present in brain or spinal cord.

Factors influencing the course of virus infection. There was a direct correlation

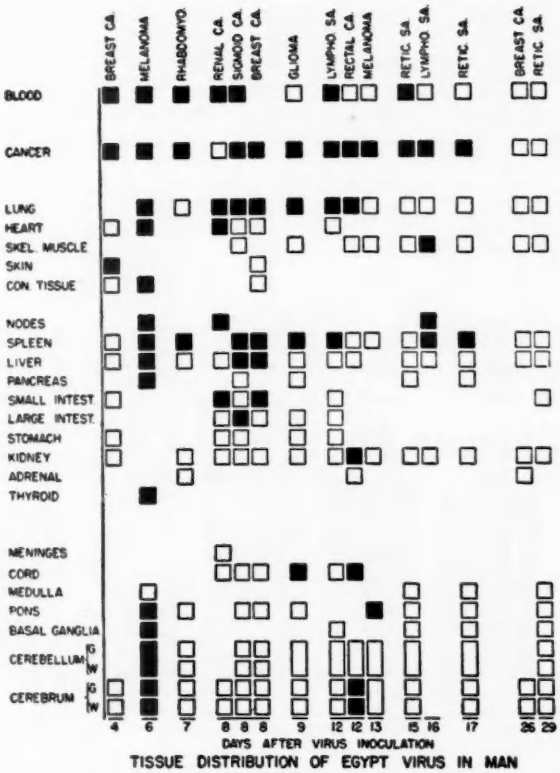

FIG. 3. Distribution of Egypt 101 virus in tissues obtained at autopsy of 14 patients who died within one month after virus inoculation. Black square indicates virus present. White square indicates virus absent.

between persistence of viremia and severity of clinical illness (Fig. 4). Protracted viremia and severe illness due to virus was more common in patients having lymphomatous disease. (This term is loosely used throughout this paper to include leukemia, Hodgkin's disease and thymoma, as well as lymphosarcoma). Six (75 per cent) of the eight patients with lymphomatous diseases had viremia persisting longer than 12 days and 62 per cent had encephalitis. This is in contrast to 3 per cent and 6 per cent respectively among patients with all other types of neoplastic disease, (Tables 3 and 4). There was no apparent relationship between other types of neoplastic disease and duration of viremia or severity of illness (Table 1).

The titer of virus inoculum had no apparent influence upon either persistence of viremia or severity of clinical reaction. (Figs. 5 and 6).

In the breakdown of data by age and sex in Tables 3 and 4, patients with lymphoma have been excluded. The slight differences in persistence of viremia

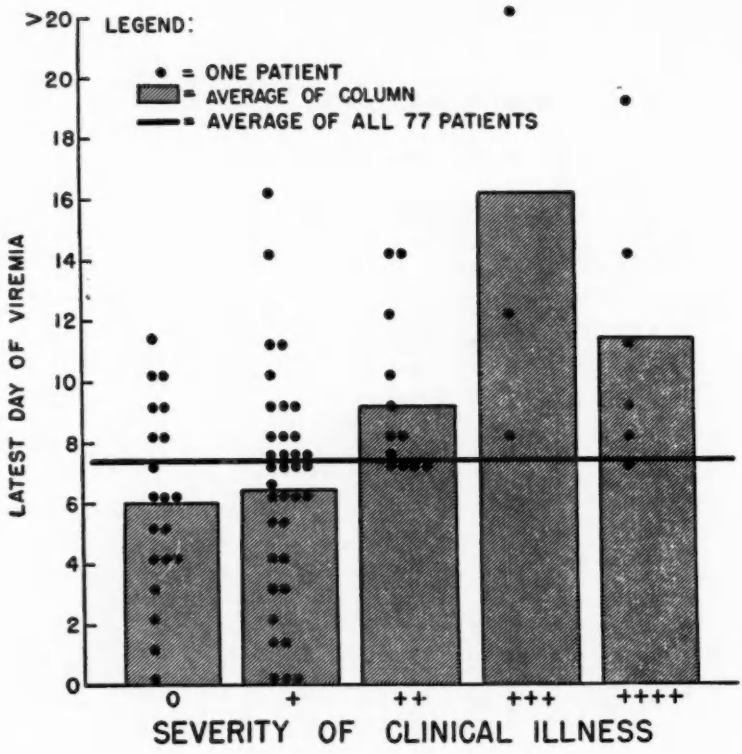

FIG. 4. Positive correlation between persistence of viremia and clinical severity of Egypt 101 infections.

and severity of virus illness between the sexes are probably not statistically significant. However, there is a slightly greater persistence of viremia and a slight tendency to more severe clinical illness among males which suggests that the male may be more prone to virus infection, even as he is to many neoplastic diseases.

Analysis of viremia and clinical severity according to age groups shows no significant differences, but the small number of patients in the old and young groups prohibits definite conclusions.

Chick embryo virus preparations were used for inoculation of eight patients (including F. B., O. F., G. W. F., and R. I. of Fig. 1). The egg and mouse preparations did not differ in infectivity for man as judged by persistence or height of viremia, or severity of clinical illness.

TABLE 3

Relationship of persistence of Egypt 101 viremia to age, sex, and reticulo-endothelial dysfunction

	PATIENTS	LATEST DAY OF VIREMIA				
	(Number)	0	1–2	3–5	6–12	>12
		(Per cent of patients)				
All patients	84	5	6	17	63	10
Lymphomas	8	13	0	0	13	75
Other cancers	76	4	7	19	67	3
Males*	46	4	2	17	72	4
Females	30	3	13	22	61	0
Age* <30	6	17	0	17	50	17
Age 30–60	56	2	7	16	73	2
Age >60	14	7	7	32	53	0

* In the presentation of data by age and sex, patients with reticulo-endothelial neoplasia (lymphoma, thymoma, and leukemia) have been excluded to eliminate the influence of reticulo-endothelial dysfunction. When such patients are included in the calculations, however, the resulting percentages are not markedly changed.

TABLE 4

Relationship of clinical severity of Egypt 101 virus infection to age, sex, and reticulo-endothelial dysfunction. (See text for explanation of grading.)

	PATIENTS	SEVERITY OF CLINICAL ILLNESS				
	(Number)	0	+	++	+++	++++
		(Per cent of patients)				
All patients	76	25	47	16	4	8
Lymphomas	8	0	37	0	25	37
Other cancers	68	28	48	17	2	4
Males*	43*	23	51	19	0	7
Females	25	35	45	15	4	0
Age* < 30	6	16	67	16	0	0
Age 30–60	49	28	43	22	0	6
Age > 60	13	29	63	0	7	0

* See footnote (*) of Table 3.

Route of inoculation was other than intramuscular in only two patients (not included in any of the above tabulations). These were inoculated intraperitoneally. Both patients had ascites due to ovarian or pancreatic adenocarcinoma. The intraperitoneal route was chosen because their cancer appeared to be

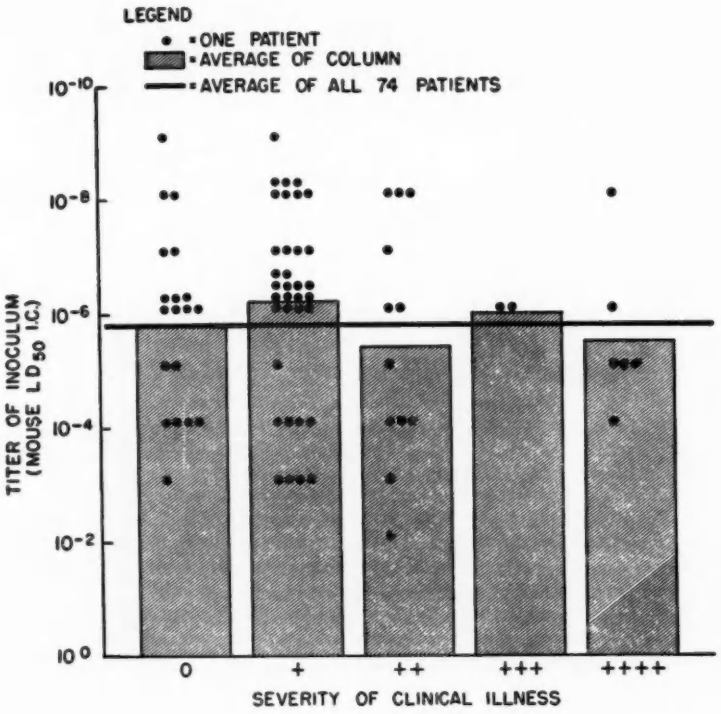

FIG. 5. Lack of correlation between inoculum size and clinical severity of Egypt 101 virus infection.

limited to the peritoneal cavity. One patient, E. B., 73, F, had viremia for three days, and a + febrile reaction. The other patient, M. H., 44, F, had a + febrile reaction and virus was recovered on day 6 only. No conclusions should be attempted from these two patients. In previous studies (Southam and Moore, 1951) patients seemed equally susceptible to virus infection by intramuscular or intravenous routes.

Effect of virus passage. Virus was often passed from patient to patient either directly (in tumor tissue) or with a single intervening mouse passage. In such passages patients were inoculated with virus from patients having the same histopathologic type of tumor. It is conceivable that by such passage a virus might become so adapted as to cause greater destruction of a given tumor type, as has been accomplished in experimental mouse tumors (Moore, 1952). (It was appre-

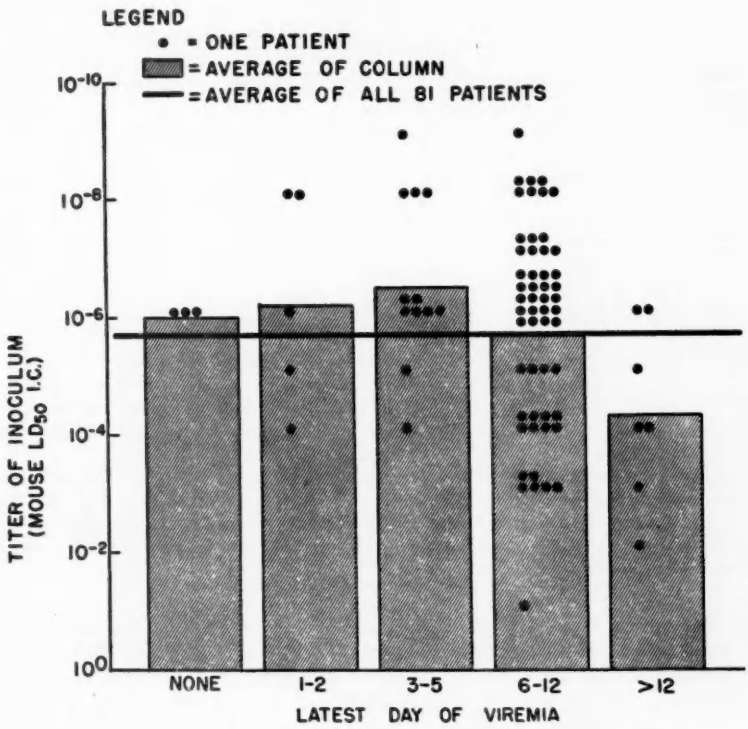

FIG. 6. Lack of correlation between inoculum size and persistence of viremia

ciated, however, that there is not necessarily any biochemical similarity between tumors of similar histologic type from different patients, and it seems probable that biochemical identity is necesary for a specific type of adaption.) These passage data are presented here in part (Tables 5 and 6) for analysis of possible changes in virulence resulting from rapid human passage. There was no change in virulence, as judged by duration of viremia or severity of clinical reaction, as a result of 5 consecutive passages in patients with lymphomatous diseases or 11 passages in patients with adenocarcinoma of gastrointestinal tract origin. Neither was there any apparent increase in oncotropic or oncolytic capacity of the virus. The most noteworthy observation is the marked variation in reaction of individuals receiving identical inocula (e. g., third passage group, Table 5; ninth passage group, Table 6).

TABLE 5

Passage protocol in reticulo-endothelial tumor group

HUMAN PASSAGE	PATIENT†	CLINICAL REACTION	VIREMIA	ONCOTROPISM	ONCOLYSIS
1	EC 35F R	++++	1-28	Yes	Yes
2	BC 31F HD	++++	1-14	Yes	Yes
3	AD 22M T	0	2-5	x	?
3	WL 66M R	++++	1-11	Select.	Yes
3	RM 43F R	++++	1-19	Yes	Yes
4	*EW 72F R	+	1-15†	Yes	?
5	RD 60M L	+++	1-12†	Yes	?

* Asterisk indicates that this patient was inoculated with human tumor suspension from preceding patient without intervening mouse-brain passage. All patients not so indicated received first mouse-brain passage from tumor of preceding patient.

† Patients are designated by initials, age, sex, and diagnosis. Abbreviation for diagnoses in this table are: R—reticulum cell sarcoma, L—lymphosarcoma, T—thymoma, HD—Hodgkin's disease. First patient inoculated with fourth mouse-brain passage stock Egypt 101. The last patient in each passage group was the source of inoculum for all patients in the succeeding passage group.

Virus infection not revealed by viremia. As shown above, viremia is usual during the early phases of Egypt virus infection. However, in two patients there was evidence that virus propagated in tissues although not detectable in the blood. Patient M. I., 21, F, had neuroblastoma. Virus was not detected at any time in blood, but tumor tissue contained virus on days 5 and 10 as indicated by paralysis and death of mice inoculated with 10 per cent tumor suspension. Patient C. K., 73, M, had squamous carcinoma. Virus was present in the blood on day 1 but not thereafter. Tumor biopsies taken on days 3, 6, and 10, all contained enough virus to kill all mice inoculated with a 10 per cent tissue suspension.

Egypt 21 isolate of West Nile virus

The Egypt 21 isolate of West Nile virus was inoculated intramuscularly into four patients. Viremia persisted for 5 to 8 days. Three patients had no clinical reaction other than slight fever. The other patient developed suggestive evidences of encephalitis (see case abstract, patient P. K., 73, M). Spinal fluids from this patient and two others were studied during infection with Egypt 21 virus, and virus was not recovered (Table 2).

Egypt 19 isolate of West Nile virus

The Egypt 19 isolate of West Nile virus was inoculated into three patients. The inoculum was third or fourth mouse brain passage, but titered less than 10^{-3}. In one patient viremia was present on days 1 through 3 only. In the other two, virus was first detected on day 3 and persisted through day 8. Virus titers in

TABLE 6

Passage protocol in gastrointestinal adenocarcinoma group

HUMAN PASSAGE	PATIENT†	CLINICAL REACTION	VIREMIA	ONCOTROPISM	ONCOLYSIS
1	GWF 50M C	+	2–11	Yes	Yes
2	OF 65M C	+	2–11	Select.	0
3	MM 54M C	++	1–10	Select.	Yes
4	JP 63M C	+	3–10	x	0
4	RM 45M C	+	1–7	x	0
4	AP 62M C	+	1–7	Select.	?
4	GR 49M P	0	1–7	x	?
4	EG 53M C	+	1–6	Select.	Yes
4	JS 40M C	++	1–8	Yes	?
4	HB 59F C	+	1–7	Yes	0
5	DB 62M S (From AP 62M)	0	1–8	x	0
5	DT 64M C	+	1–5	x	0
6	OB 45F C	+	1–6	x	0
6	BT 44F C	+	1–7	x	0
6	MS 43M C	+	1–5	Yes	0
6	DK 57F C	x	1–7†	Select.	x
7	LR 68M S	+++	1–8	x	0
7	SW 47M C (From MS 43M)	+	1–7	x	0
7	LP 63M C	+	1–9	Yes	0
8	LV 40M C	0	1–6	Yes	0
9	VB 55F P	0	2	x	0
9	YT 57F C	+	1–8	x	0
9	HT 44F C	0	1–3	x	0
9	AD 42M S	++++	1–7	x	0
9	MS 39M C	++	1–7	Yes	0
10	*HM 59M C	0	1–8	Yes	0
10	HK 60M C	+	1–7	Select.	0
11	AG 59M C	++	1–7	Yes	0
11	HK 47M C	0	1–6	Select.	0

* Asterisk indicates that this patient was inoculated with human tumor suspension from the last patient in the preceding passage group without intervening mouse-brain passage. All patients not so indicated received first mouse-brain passage from human tumor.

† Patients are designated by initials, age, sex, and diagnosis. Diagnosis in each case is adenocarcinoma. Site of origin is indicated by the following symbols: C—colon (includes cecum and rectum), S—stomach, P—pancreas. The first patient was inoculated with fourth egg passage stock Egypt 101. The last patient in each passage group was the source of inoculum for all patients in the succeeding passage group, with 2 exceptions for whom the source is specifically designated.

bloods rarely exceeded 10^0. One patient lived more than three weeks after virus inoculation. There was no clinical reaction to the virus. One patient died five days following inoculation. Virus was present only in the spleen, of the many tissues studied after autopsy. There was no virus in tumor or in any portion of brain or cord. The third patient died on day 10. A tumor biopsy on day 5 was negative for virus when blood was positive. Virus was not detected in tissues obtained at autopsy.

DISCUSSION

Clinical importance of West Nile virus: West Nile virus has been isolated from human sera collected in Uganda (Smithburn *et al.*, 1940), Egypt (Melnick *et al.*, 1951; Taylor, 1953), and Israel (Bernkopf; Goldblum, 1953), and Culex mosquitoes in Egypt (Taylor and Hurlbut, 1953). It has been demonstrated that under laboratory conditions mosquitoes (Taylor and Hurlbut, 1953; Philip and Smadel, 1943; Kitaoka, 1950) can transmit the virus from infected to healthy mice. In addition, anti-West Nile antibodies have been demonstrated in sera collected from natives of India (Smithburn, 1953). All of the available information concerning the course of naturally-occurring infection indicates that West Nile virus causes a mild illness in man, but infection may exhibit an epidemic pattern (Goldblum, 1953). The present study indicates that West Nile virus is, in occasional patients, able to cause severe encephalitis or encephalomyelitis. It seems possible, therefore, that West Nile virus will occasionally, under natural conditions of transmission, cause significant clinical illness in man. The possibility of virus mutation in the direction of increased neurotropism makes it conceivable that this virus could at any time attain real clinical importance. It should be noted that anti-West Nile antibodies have never been observed among approximately 80 patients and staff studied at this hospital prior to the experimental inoculation of the virus. Most of these patients came from the New York City area, but residents of Pennsylvania, Virginia, Texas, and Washington are included in the series.

The problem of diagnosis. The results of the present studies explain why it is so often difficult to make a definitive diagnosis in certain virus diseases. It is apparent from the data presented that virus illness would not have been suspected during the period of viremia in most of the patients studied, and after symptoms of central nervous system involvement appeared, virus would rarely have been recovered from blood or spinal fluids. Attempts to diagnose the infection by demonstration of virus in urine, stool or throat washings would be futile at any period. The few patients in whom virus propagation was demonstrated by study of tissue biopsies, but who had no detected viremia, would remain undiagnosed even if all usual diagnostic methods were initiated promptly. Diagnosis by antibody studies (no data presented in this paper except the curves in Figures 7 through 18) would not be diagnostic in most patients until at least three weeks following virus inoculation, and only if these specific etiologic agents were correctly suspected. It is difficult to imagine what type of technics might be introduced which would solve this diagnostic dilemma.

Relation of host and virus factors to clinical reaction. The importance of inherent virus pathogenicity in the determination of clinical disease is seen by comparing previous results (Southam and Moore, 1951), using high mouse passage West Nile virus and the present results with virus recently isolated from man. There is ample evidence that passage of a virus in another species changes its pathogenicity for the original host. This may explain the low infectivity of the high mouse passage virus.

The importance of individual host susceptibility is indicated in the present study by the tremendous differences in clinical response which occurred among patients inoculated with identical virus material. The integrity of the reticulo-endothelial system is seen to be one factor of major importance in such individual susceptibility, but it is also apparent that there are additional host factors which we have not been able to measure, for in the present series of patients the most severe reaction occurred in a patient (J. E., 64, M) who had relatively little neoplastic disease, a normal blood picture, and no evidence of impairment of function of liver, spleen, or lymph nodes. It also appears that antibody forming ability is not the major factor in the determination of individual clinical response to virus exposure, since the patient with most severe encephalitis (J. E., 64, M, Fig. 7) had an apparently normal rate and degree of antibody response, whereas patient G. R., 49, M (Fig. 18) who showed no clinical reaction to the virus infection had a very poor (or slow) antibody response.

The known participation of the reticulo-endothelial system in both cellular and humoral defense mechanisms seems an adequate explanation for the increased susceptibility to virus infection of patients with lymphomatous disease. It should be appreciated, however, that there are two known causes of reticulo-endothelial system dysfunction in patients with lymphomatous disease: first, the neoplastic process with its production of abnormal cells and consequent invasion and replacement of normal reticulo-endothelial structures; and second, the decreased number of reticulo-endothelial cells caused by the therapeutic use of x-ray, "radio-mimetic" chemicals such as nitrogen mustards, and cortisone or related hormones. There are no data from the present studies, and no published work of which the authors are aware, that permit distinction between these two causes. Neither has it been possible to ascertain the relative importance of cellular defense and humoral defense inadequacies.

Significance of virus tropism. West Nile virus, and many other viruses, have been classified as neurotropic on the basis of their effect upon the central nervous system of mice when inoculated intracerebrally. The present study makes one question whether central nervous system damage, under such experimental conditions, can be accepted as an indication of potential encephalitogenic capacity for man. While it has been fully demonstrated that this virus can be encephalitogenic in man, it is equally apparent that virus propagation may occur over a period of many days without any clinical evidence of central nervous system involvement, and without apparent virus invasion of central nervous system (as judged from the spinal fluid and autopsy studies).

The fact that, among normal tissues, Egypt virus localized most frequently

and persistently in spleen and lymph nodes suggests an affinity for reticulo-endothelial cells. The localization of virus in neoplastic tissues has been (Southam and Moore, 1952) and will be further discussed elsewhere.

It is worthy of note that the presence of this virus in a tissue (normal or neoplastic), even in high concentrations, was not usually accompanied by microscopically detectable cytopathologic changes in that tissue (Newman and Southam, 1954). It has also been demonstrated (unpublished data) that Egypt 101 virus and the original West Nile virus can grow for long periods of time in tissue cultures of certain human tissues without producing obvious cytopathology.

Antineoplastic effect. It is not the purpose of the present publication to present a detailed analysis or discussion of the antineoplastic effects attributable to the Egypt isolates of West Nile virus. The occurrence of definite or suggestive objective regressions in tumors is recorded in Table 1. Regression of a mediastinal reticulum cell sarcoma mass is mentioned in the case history of one patient (R. M., 43, F). It was previously reported (Southam and Moore, 1952) that a few objective tumor regressions had occurred in patients with adenocarcinoma of gastrointestinal origin and in patients with tumors of reticulo-endothelial origin. It was concluded at that time that since regressions occurred coincidental with or shortly following the presence of virus in tumor tissue, they were presumably due to the virus but were not of a sufficient degree nor of sufficient duration to be practical therapy for advanced cancer. It was also concluded that the oncolytic effect of viruses is of considerable fundamental interest, and might be of practical therapeutic value if more effective viruses are discovered, if more sensitive tumors can be selected for treatment, or if the present viruses can be so adapted as to cause increased oncolysis. These previous conclusions remain valid.

SUMMARY AND CONCLUSIONS

Ninety patients with advanced neoplastic diseases were inoculated intramuscularly with the Egypt 101 isolate of West Nile virus, as part of an experimental clinical evaluation of the antineoplastic effect of viruses. The Egypt 19 and Egypt 21 isolates of this same virus were inoculated into three and five patients respectively. This paper describes the clinical course and virology of the resulting infections. Reports concerning antineoplastic effects, serology, and histopathology have been, or will be, published elsewhere.

All three of the Egypt isolates of West Nile virus infected man, as indicated by the presence of virus in blood at three days or later following virus inoculation. No significant differences between the pathogenicity of the three isolates were apparent, but the trials of Egypt 19 and Egypt 21 were too few to warrant definite conclusions. Egypt 21 infection was followed by suggestive clinical evidences of diffuse encephalitis in one patient.

Virus was recovered from blood of 95 per cent of the patients inoculated with Egypt 101. Viremia persisted through day six in 73 per cent, and longer than day twelve in 10 per cent. Virus was not present in feces, urine, or throat washings. Virus was demonstrated in spinal fluid of three patients during infection with

Egypt 101 virus. All three of these patients had clinical evidences of encephalitis. Two of the virus isolations from spinal fluid occurred during the period of viremia, but virus was present in spinal fluid of one patient five days after the blood had become virus free. Spinal fluid specimens from 11 other patients did not contain virus during infection with Egypt 101 or Egypt 21 viruses, even at the time of clinical encephalitis (five patients), or during viremia (six patients).

Eighty-nine per cent of patients inoculated with Egypt 101 virus had no clinical evidence of virus disease other than fever, and temperature elevation did not exceed 3°F. in 73 per cent. Eleven per cent of patients had definite or suggestive signs of diffuse encephalitis characterized by twitching and mental confusion and one of the patients also had flaccid paralysis of extremities. Neurologic impairment was transient and recovery was complete in all patients who did not rapidly succumb to their neoplastic disease.

There was a direct correlation between the duration of viremia and the severity of clinical illness. Patients with neoplastic disease of reticulo-endothelial cell origin had a striking predisposition to prolonged viremia and increased severity of clinical illness. Age and sex showed no significant correlation with either duration of viremia or severity of the clinical illness. There was marked individual variation in duration of viremia and severity of illness among patients who received identical inocula.

The distribution of virus in tissues obtained at autopsy was studied in 15 patients who died within four weeks after inoculation of virus. Virus was demonstrated in neoplastic tissue of various histopathologic types. The normal tissues most frequently containing virus were spleen, lymph nodes, liver, and lungs, suggesting distribution determined by reticulo-endothelial cells. Central nervous tissue was only occasionally found to contain virus and there was no consistent anatomical localization of virus within the central nervous system.

CASE ABSTRACTS

Patient J. E., 64, male, muco-epidermoid carcinoma of the parotid with pulmonary and subcutaneous metastases, Fig. 7: Egypt 101 virus was inoculated I.M. on May 2, 1952 (2.0 cc. of 20 per cent mouse brain suspension). The virus inoculated was first mouse brain passage after recovery from epidermoid carcinoma biopsy from another patient, and had been through a total of three similar human passages (with intervening mouse passages) prior to inoculation into this patient. In none of the previous human passages had there been any reaction other than fever. There was an abrupt febrile spike to 103°F. at 24 hours after virus inoculation and to 104.4 at 48 hours in spite of large doses of penicillin and streptomycin. No explanation for the fever, other than the virus infection, could be established, although such an abrupt febrile response had not been seen in any other patient. On day 4 the patient complained of generalized headache and dizziness. He was mentally clear but very apprehensive. Neurological examination was negative. By day 7 there was involuntary twitching of the jaw, hands and forearms when at rest. This could be prevented by the patient at will. The sensorium was still intact and there was still no objective sign of central

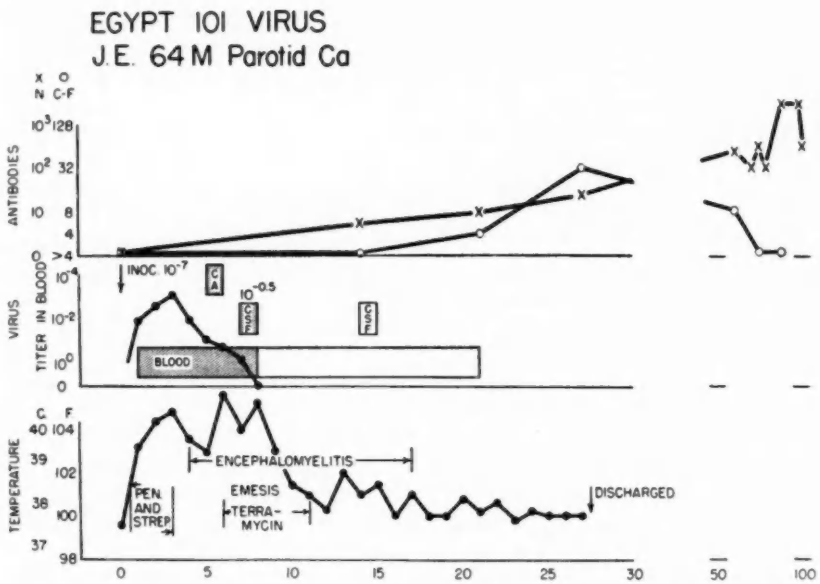

FIG. 7. Egypt 101 virus infection in patient J. E., 64, M, in whom definite but transient encephalitis with paralysis occurred.

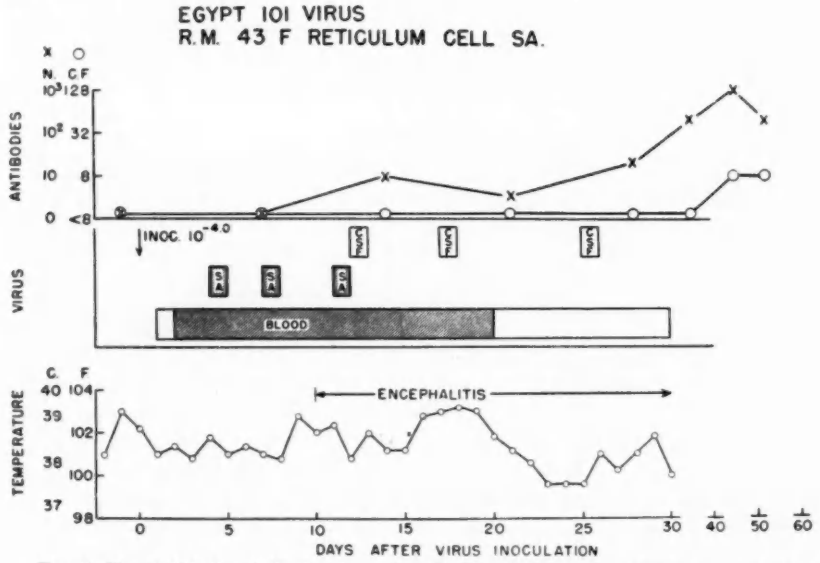

FIG. 8. Egypt 101 virus infection in patient R. M., 43, F, in whom definite encephalitis ensued.

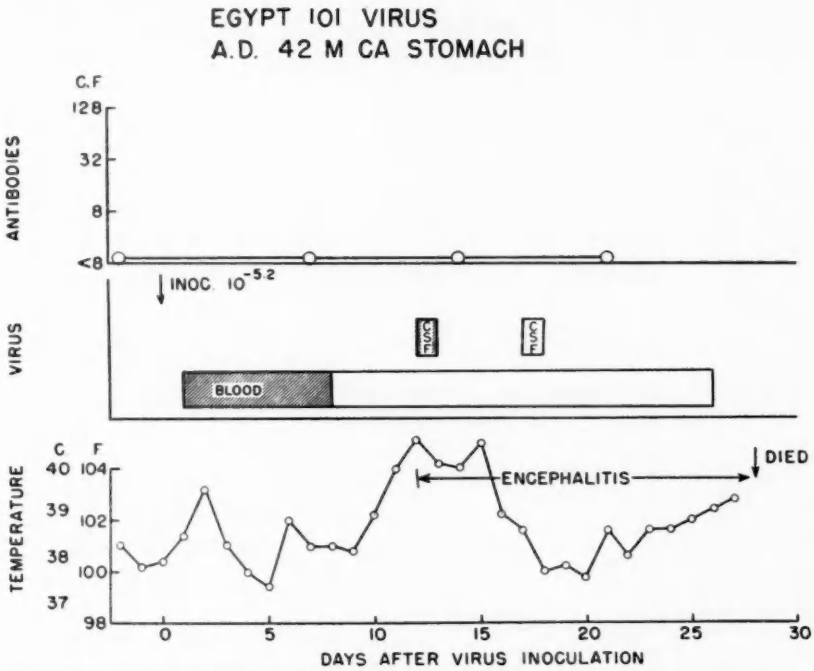

FIG. 9. Egypt 101 virus infection in patient A. D., 42, M, in whom definite clinical indications of encephalitis occurred. Note that cerebro-spinal fluid contained virus (indicated by cross-hatched block) five days after blood became virus-free.

nervous system disease other than the twitching. The patient still felt "light headed" but his headache was decreasing. Spinal tap at this time showed 40 mononuclear cells per cubic millimeter and initial pressure was 85 millimeters of water. Spinal fluid chemistries were normal. Virus was recovered from spinal fluid on this date. Its identity was esablished by neutralization test. On day 8 the patient had an episode of hematemesis. Guaiac test was strongly positive. This was the only episode of vomiting. The patient had no neoplastic involvement of the gastrointestinal tract. Possibly the hematemesis was a result of the virus infection but no such signs have been observed in any other patient. On day 10 the temperature had returned to normal, but there was abrupt development of almost complete flaccid paralysis of both lower extremities. Deep tendon reflexes were still intact and equal. Babinsky was negative. Sensation was intact. Uretheral and rectal sphincters functioned normally. On day 14 cerebro-spinal fluid showed protein elevated to 92.4 mg. per cent, but there was no other abnormality and virus was no longer present. On day 16 he complained of pain in the left shoulder and on day 18 was found to have a definite paresis of the left shoulder girdle (but not of the arm, forearm, or hand). By day 21 muscular strength in legs and

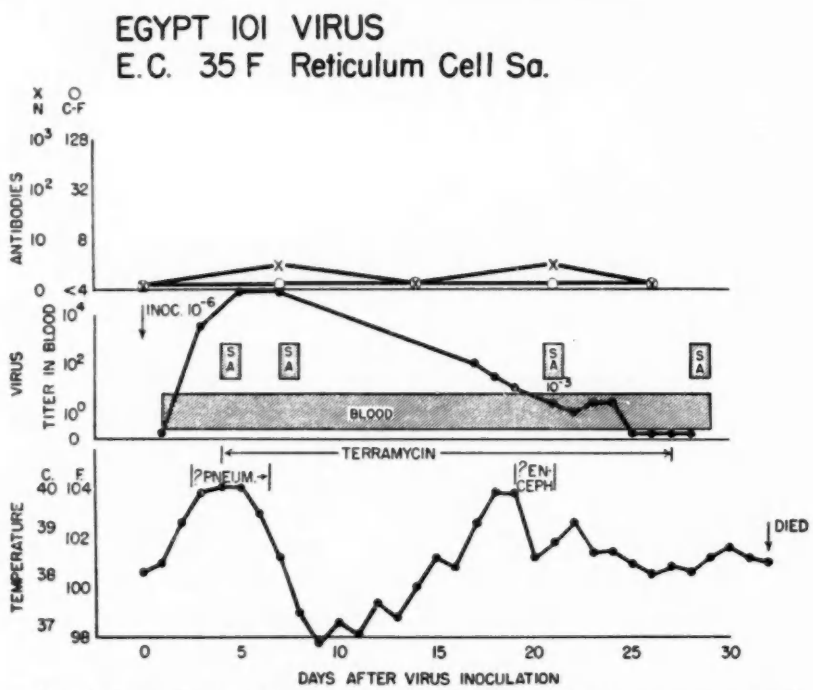

FIG. 10. Egypt 101 virus infection in patient E. C., 35, F, in whom suggesti ve clinica indications of transient encephalitis occurred.

arm was definitely improving and by day 24 the patient was able to walk with support. Recovery of strength was rapid. By two months after virus inoculation muscle strength was intact except for some weakness of the extensors of the thighs, and the patient was able to climb two flights of stairs. There was no apparent progression of this patient's neoplastic disease during the period of virus disease or for about six months thereafter, but neither was there any regression, so this patient is tabulated as showing no antitumor effect. The patient is still alive and shows no neurologic sequelae 12 months after virus inoculation. The course of virus infection in this patient was highly suggestive of acute anterior poliomyelitis, and differs from every other patient in this series by the disturbance of motor function. The possibility that motor impairment was due to coincidental infection by another virus, or that another virus infection was activated by the Egypt 101 virus inoculation, is worthy of consideration, but the fact that Egypt virus was recovered from spinal fluid during the initial febrile period makes it highly probable that the entire picture in this patient was due to the Egypt 101 infection.

Patient R. M., 43, female, reticulum cell sarcoma, Fig. 8: On August 15 this patient was inoculated with a mouse brain preparation of Egypt 101 virus which

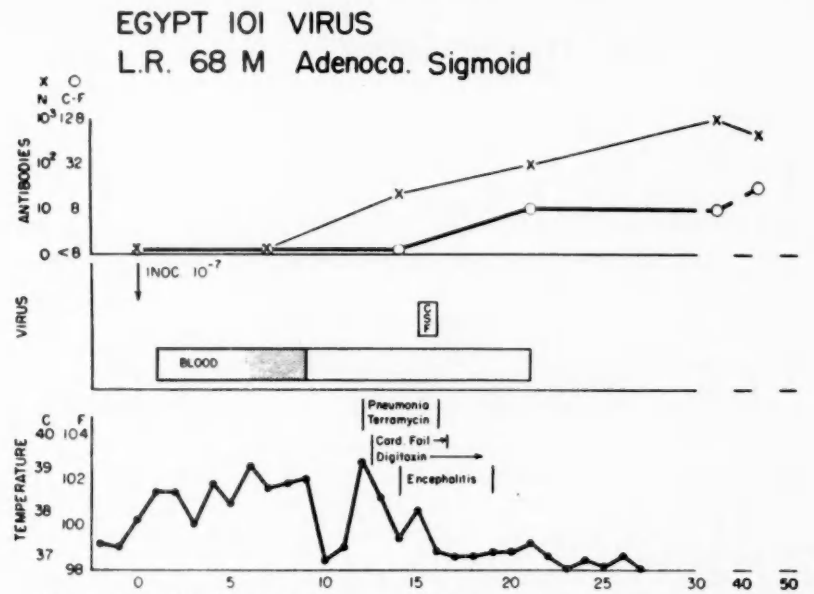

FIG. 11. Egypt 101 virus infection in patient L. R., 68, M, in whom suggestive clinical indications of transient encephalitis occurred.

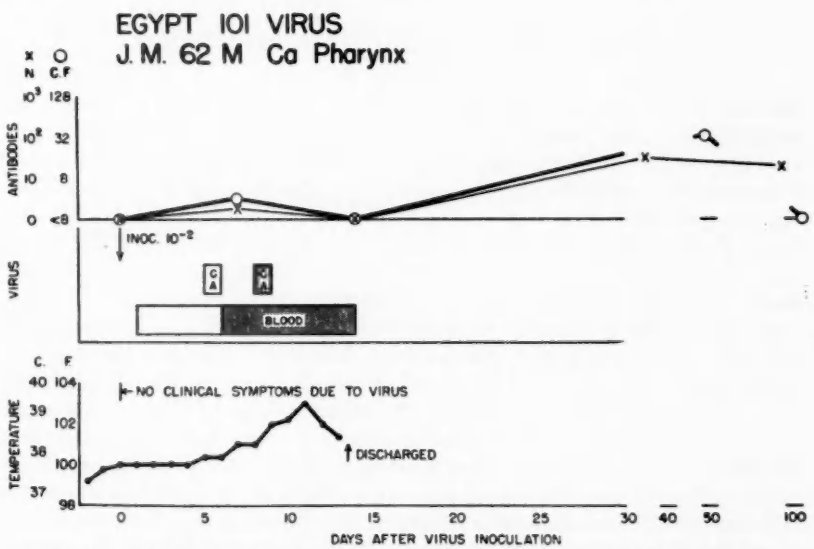

FIG. 12. Egypt 101 virus infection in patient J. M., 62, M. The only clinical reaction was fever. Note the late onset of viremia.

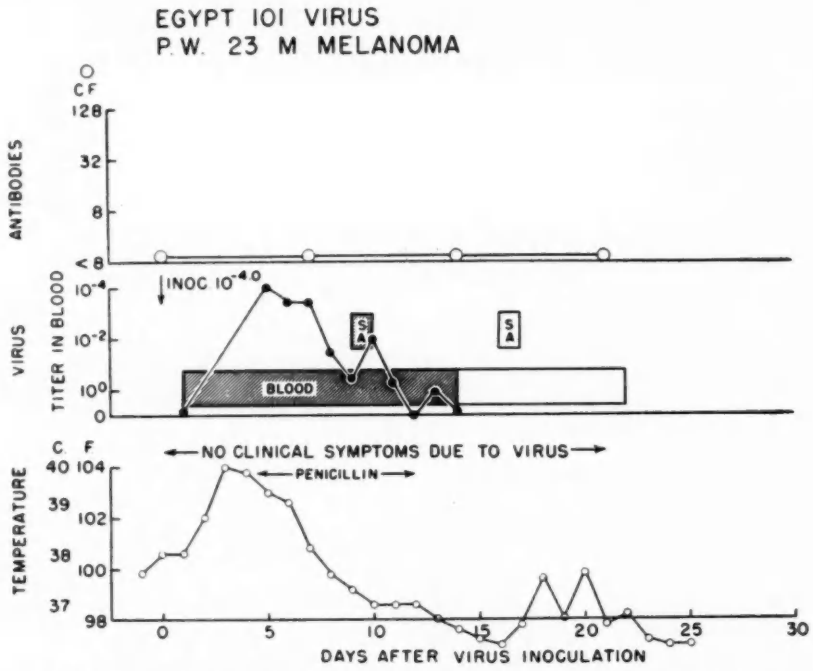

FIG. 13. Egypt 101 virus infection in patient P. W., 23, M. The only clinical reaction was fever.

had previously been passed through one patient with reticulum cell sarcoma and one with Hodgkin's disease and had caused tumor regression in each (Table 5). This patient had massive adenopathy and a mediastinal mass which regressed dramatically as judged by physical examination and chest roentgenograms, starting five days after virus inoculation. There was no recurrence during her remaining seven weeks of life. This tumor regression seems attributable to the virus. A more complete presentation of case data is planned. This patient had viremia through day 19. Titrations were not done, but on days 1 and 2 undiluted blood contained less than an LD_{50} dose of virus, and thereafter the virus content rose abruptly as indicated by deaths in all inoculated mice with paralysis and death appearing as early as five days after the mice were inoculated. The virus concentration probably diminished after the first ten days as evidenced by occurrence of paralysis and death at a somewhat longer time after mice were inoculated. On the eighteenth and nineteenth days following virus inoculation undiluted blood again was less than an LD_{50}. Scattered deaths also occurred in mice inoculated with blood taken as late as twenty-four days after the patient's inoculation. The patient's temperature reaction (Fig. 8) is difficult to interpret

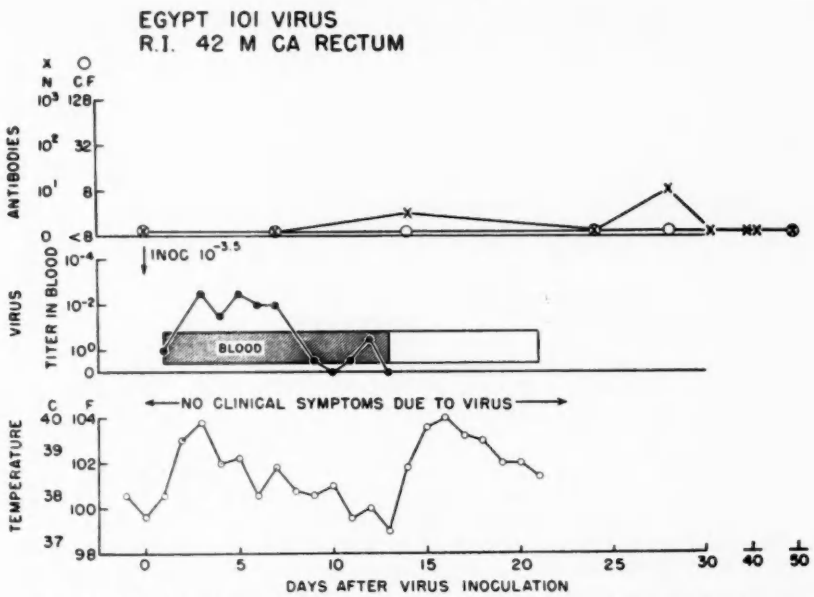

FIG. 14. Egypt 101 virus infection in patient R. I., 42, M. The only clinical reaction was fever.

because of the presence of fever preceding virus inoculation. There was, however, an increase above preceding temperature levels on days 9 through 11, and day 16 through day 19. Beginning on day 10 the patient evidenced increasing restlessness and emotional lability, and twitching of the hands and occasionally of the feet. On day 14 she was semi-comatose. By day 19 she was "a little brighter" as indicated by responsiveness to speech, but was still restless, and was still having some twitching of the extremities. For the next two weeks the patient remained in a semi-comatose condition most of the time. On day 35 the patient was described as fairly lucid, well-oriented, and able to converse. She was taking small amounts of a full fluid diet by mouth. This improved mental condition persisted for the following week. On the forty-third day after virus inoculation, she was described as being quite alert and conversing logically. On the forty-fourth day, however, she had an abrupt change for the worse, becoming completely irrational and thrashing in bed. Shortly thereafter she became quiet and remained in an almost complete coma until her death on the fifty-fifth day after virus inoculation. Throughout the entire course of this patient's viral illness, there was no change in objective neurologic signs other than the twitching. Specifically, there was no anesthesia, no paralysis, no cogwheeling, and no stiff neck. Lumbar puncture was performed on days 12, 18, and 25 following virus inoculation. Virus was not recovered from any specimen. Pressure was elevated to 260 mm. on the

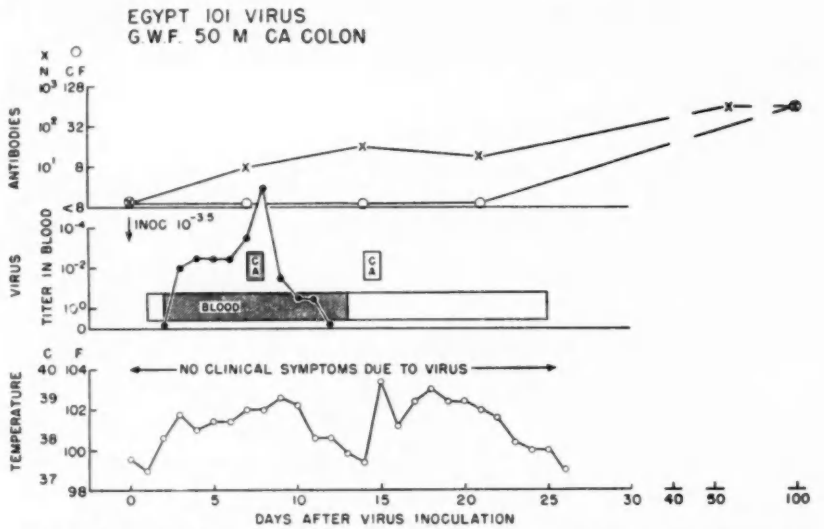

FIG. 15. Egypt 101 infection in patient G. W. F., 50, M. This patient's clinical course was characterized by irregular bouts of fever both prior to and subsequent to Egypt virus infection.

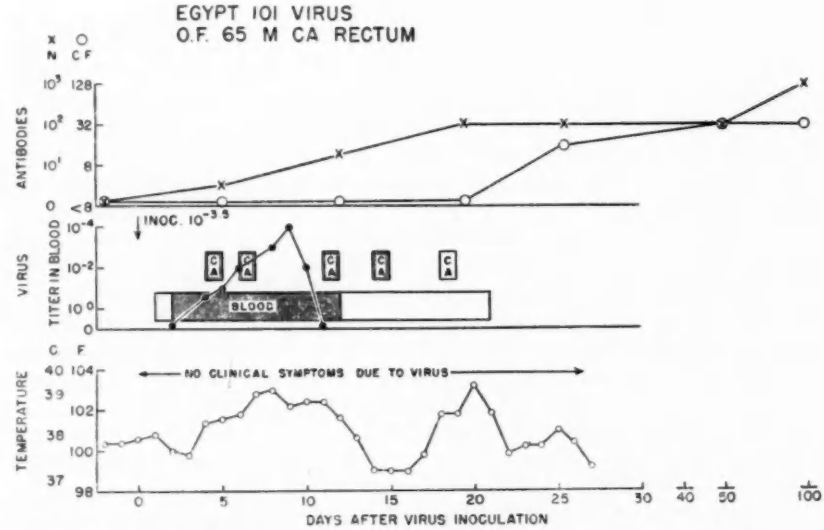

FIG. 16. Egypt 101 virus infection in patient O. F., 65, M. The only clinical reaction was fever. Note persistence of virus in tumor tissue at 3 days after blood became virus-free.

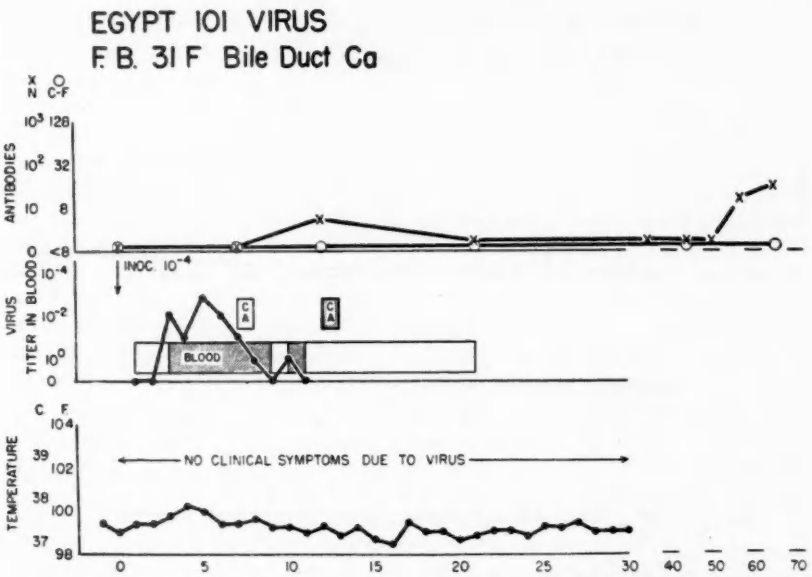

FIG. 17. Egypt 101 virus infection in patient F. B., 31, F. Note virtual absence of fever (graded as an "O" clinical reaction), and persistence of virus in tumor tissue (from liver) taken two days after blood became virus-free.

first of these days but was not abnormal thereafter. There was no increase in leukocytes in any of the three specimens. Proteins increase progressively to a maximum of 63.6 mg. per 100 cc. in the third specimen (day 24).

Patient E. C., 35, female, reticulum cell sarcoma, Fig. 10: Egypt 101 virus (2.0 cc. of 20 per cent suspension) was inoculated I.M. on June 15, using fourth mouse-brain passage from the original isolation. Four days later her temperature rose to 104°F., but this was accompanied by clinical signs of pneumonia and the temperature promptly fell to normal following terramycin treatment (Fig. 10). At the beginning of the third week following virus inoculation her temperature again rose in spite of continued terramycin and again reached 104° on the eighteenth post-inoculation day. On days 19 and 20 the patient was unusually drowsy and was observed to have occasional twitching of the hands. There were no changes in objective neurologic examination. The temperature fell by day 20 and thereafter it stayed at about 102°. The patient became mentally clear and had no further signs of central nervous system impairment. She died 52 days after virus inoculation, due to respiratory insufficiency caused by pulmonary sarcoma and effusion. The period of fever with drowsiness and twitching was regarded clinically as suggestive of transient encephalitis and is tabulated as a +++ clinical reaction to virus infection. This clinical impression was confirmed by microscopic evidences of encephalitis at autopsy. This patient showed marked decrease in size of palpable tumors during the period of virus infection, and this

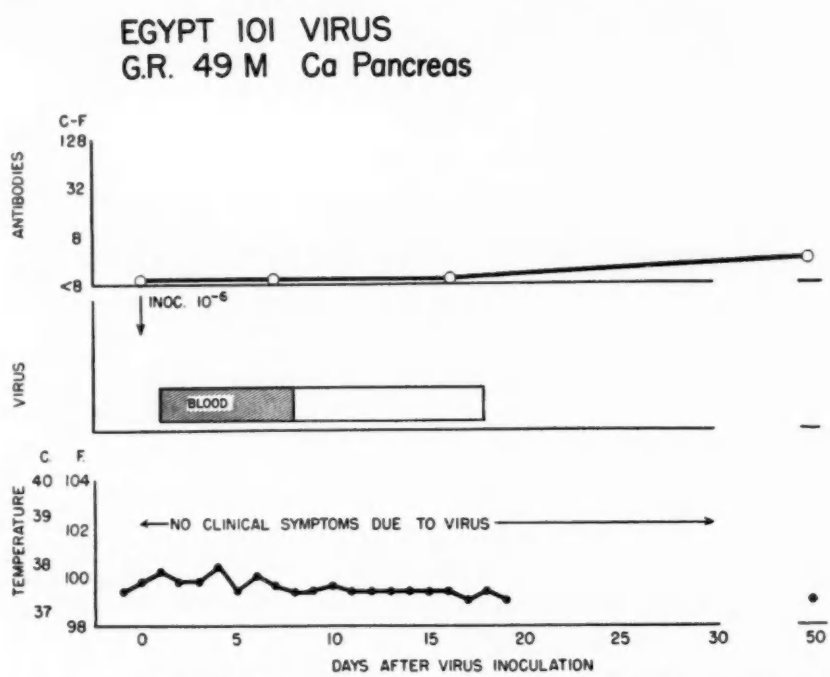

FIG. 18. Egypt 101 virus infection in patient G. R., 49, M. Note virtual absence of fever (graded as a "O" reaction).

was accompanied by extensive necrosis in the biopsied lymph nodes. Photomicrographs are presented in a previous publication (Southam and Moore, 1952). Virus was present in all of the biopsied lymph nodes and the period of viremia persisted for 28 days. During the week prior to death there was a very rapid regrowth of tumors.

Patient L. R., 68, male, adenocarcinoma of colon with pulmonary metastases, Fig. 11: Egypt 101 virus was inoculated I.M. (3.0 cc. of 10 per cent mouse-brain suspension, 7th human passage, see Table 6) on July 31, 1952. Temperature had been as high as 100.4° on the day of and preceding virus inoculation. During the following nine days the daily maximum fluctuated between 101 and 103°F. On days 10 and 11 temperature was normal but on day 12 it spiked again to 102.8°. Gantrisin had been administered constantly from before virus administration through day 14 because of known cystitis and indwelling catheter, necessitated by prostatic hypertrophy. On day 12 the patient was also started on terramycin and was digitalized because of increased respiratory difficulty, bilateral leg edema and increasing size of liver. It was suspected that the patient had bronchial pneumonia and cardiac failure superimposed upon his neoplastic disease. Response of fever to the treatment was good (Fig. 11), but on day 15 he became

semicomatose, developed twitching and spasticity of extremities, and hyperactive deep tendon reflexes. Cerebro-spinal fluid contained 17 mononuclear cells per cubic millimeter and the protein was elevated to 63.8 mg. per cent. Pressure and sugar were normal. Virus was not present. By day 17 the patient showed definite recovery from what was regarded clinically as encephalitis. By day 20 the patient was afebrile, ambulatory, and showed no sequelae of encephalitis, cardiac failure, or pneumonia. The patient died due to progressive pulmonary metastases about two months later. Autopsy showed no signs of central nervous system pathology. There were extensive pulmonary and hepatic metastases. This patient's reaction to virus infection was graded as +++. There was a definite regression in liver size during the three weeks following virus infection, but this was considered to be a result of correction of cardiac decompensation rather than regression of metastatic tumor in the liver, so this patient is tabulated as showing no evidence of tumor regression.

Patient P. K., 73, male, adenocarcinoma of rectum with uremia due to recurrent tumor. This patient had a tumor mass compressing the bladder, and no evidence of renal function by intravenous pyelogram. His blood urea nitrogen was 90.9 mg. per 100 cc. It was felt that the patient's condition would not permit surgery, and the patient's family requested experimental methods of treatment. Fourth mouse-brain passage Egypt 21 (3.0 cc. of 10 per cent suspension) was inoculated I.M. September 13, 1952. Viremia was demonstrable for the next five days. There was no abnormality of temperature until day 13 when it increased abruptly to a maximum of 103°F, and remained elevated until day 19 in spite of constant treatment with penicillin and streptomycin and subsequent intravenous administration of aureomycin. These antibiotics were given because of the development of clinical and x-ray evidence of pneumonia at the site of the previous postoperative atelectasis. On day 12, the day before start of fever, this patient complained of headache, seemed unusually apprehensive, and vomited several times. On the following day, as his temperature rose, he appeared slightly confused, and had occasional twitching movements of the arms. Spinal fluid specimens on days 13 and 16 did not contain virus, and protein was within normal limits, but there were 18 and 20 mononuclear cells per cc. (Table 2). Deep tendon reflexes were active throughout, but not abnormal. The patient resisted passive movements, but this appeared voluntary. The neck was not stiff. During the next few days the patient became somewhat more alert, and vomiting and twitching ceased. There was no further complaint of headache. For the next several days the patient's condition seemed to be improving slightly but on day 19 he became less responsive, developed peripheral vascular collapse and depressed respiration. The patient survived only a few hours after these changes. Permission for autopsy was refused. The clinical impression was that this patient probably had virus encephalitis from which he was recovering at the time of his death. This is tabulated as a +++ clinical reaction to the Egypt 21 virus infection. However, the concurrent diagnoses of metastatic adenocarcinoma, extreme uremia, and pneumonia; and the absence of autopsy examination, make it impossible to give a definite diagnosis of virus encephalitis.

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REPORT OF CLINICAL CASE OF WEST NILE VIRUS INFECTION PROBABLY ACQUIRED IN THE LABORATORY[1]

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This report describes an infection by West Nile virus in a technician who was working with the virus in the laboratory. Evidence favors the view that the infection was acquired in the laboratory. We have found no record of a similar case in the literature.

The West Nile virus was first isolated in 1937 from the blood of a febrile woman in the West Nile province of Uganda (Smithburn *et al.*, 1940). It was again isolated in 1950 in an Egyptian village north of Cairo (Melnick *et al.*, 1951). The virus when inoculated intracerebrally into Swiss mice produces an encephalitis and is highly fatal.

Southam and Moore (1952) in a study of the effect of the virus on neoplasms, experimentally infected 68 humans with a strain of West Nile virus (Egypt 101). Four patients developed definite signs of diffuse encephalitis and two patients showed transient signs of encephalitis.

Case report: D. T., a 35 year old white male, virology technician, was first seen on August 15, 1952, complaining of severe fatigue, malaise, generalized muscular pain of 48 hours duration. In addition he had had a slight cough and slight pain on the movement of both eyes. His chief complaint was a constant moderately severe pain of both calves and thighs.

Present illness: The patient had been in good health prior to August 10, 1952, when he developed a slight sore throat. This persisted until August 13, 1952, when he developed a moderately severe dull pain in both calves and thighs. On August 14, 1952, the patient felt better during the morning but the pain in the calves, associated with fatigue and malaise, returned in the afternoon. The patient stated that at 6 p.m. on this day, his temperature was 99.8°F. (oral). He had been employed in the virology laboratory for 6 months.

Physical examination (August 15, 1952): Temperature 99.8°F., pulse 100, blood pressure 125/75.

General appearance: The patient is a young adult male appearing acutely ill but in no great distress.

Positive physical findings were: Chest: increased breath sounds in the right axilla. Abdomen: moderate tenderness in the right hypochondrium. Liver slightly palpable but no tenderness elicited. Lower extremities: marked tender-

[1] The opinions and assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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ness of both gastrocnemius muscles on pressure. Neurological examination: normal.

Clinical course: The patient was given complete bed rest and symptomatic treatment. The pain in the calves persisted until August 17, 1952, at which time some improvement was noted. At this time, the patient developed a dull pain between the scapulae. On August 18, 1952, the pain in the calves had disappeared but the patient still had generalized weakness, malaise and easy fatigability. The patient showed gradual symptomatic improvement but did not feel normal until August 25, 1952. During the course of the illness, the temperature remained at 99°F. (oral). Chest x-ray on August 15, 1952, was interpreted as normal.

Laboratory studies: Urinalysis: Specific gravity 1.016. Albumin: negative. Sugar: negative. Microscopic: 1 to 3 red cells per high power field. Complete blood count: R.B.C. 5,450,000 per cu.mm. W.B.C. 5,850 per cu.mm. Hemoglobin 14.5 grams/100 ml. *Differential:* Segmented forms: 48%. Lymphocytes 41%. Eosinophiles 1%. Monocytes 10%. *Liver function tests:* Zinc turbidity 7.4 units; thymol turbidity 1.5 units; phenol turbidity 13.0 units; bilirubin 0.33 mgm/100 ml.; plasma proteins 7.15 gms/100 ml.

Summary of virological and serological studies

5–16–52 (blood) West Nile Complement Fixation negative 1 to 4
8–13–52 Onset of illness
8–14–52 (blood) West Nile Complement Fixation negative 1 to 4
8–25–52 (blood) West Nile Complement Fixation positive 1 to 64
8–29–52 (blood) West Nile Complement Fixation positive 1 to 64
9–15–52 (blood) West Nile Complement Fixation positive 1 to 64

A virus was isolated from the acute phase blood taken on 8–14–52 and identified as West Nile virus. In addition a neutralization test on serum taken preceding the attack was negative while the serum taken approximately one month (9–15–52) following the illness neutralized West Nile virus.

Discussion: The clinical picture described here is that of an undistinctive febrile illness. The most impressive symptom and sign was the severe pain and marked tenderness of both gastrocnemius muscles. Since the West Nile virus has been shown to be neurotropic in mice and humans, this could be explained on the basis of a peripheral neuritis. There was no clinical evidence of a meningoencephalitis, although the spinal fluid was not examined.

The clinical laboratory studies revealed little information of significance. The leucocyte count (5,550 per cu.mm.) with the relative lymphocytosis (41%) is compatible with a virus infection. Although the liver was slightly palpable, the liver function tests were all within normal range.

The isolation of West Nile virus from the blood during the acute illness and the development of both complement fixing and neutralizing antibodies to West Nile virus following the attack clearly indicate infection with this virus.

It is of interest and perhaps of significance that this technician was engaged principally in the inoculation and passage in mice of human bloods and mos-

quitoes from which several strains of West Nile virus were isolated shortly before his illness. This suggests, as was found by Southam and Moore (1952), that freshly isolated strains of this virus may be more infectious to man than strains that have undergone many serial passages in mice.

The patient was not aware of any break in technique and the route and manner of infection is unknown. In that West Nile infection is endemic in Egypt and the virus has been found in wild-caught mosquitoes (Taylor *et al.*, in preparation) the possibility of infection having been acquired outside of the laboratory cannot be eliminated. However, in view of the occupation of this person it seems highly probable that infection was acquired in the laboratory.

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EASTERN EQUINE ENCEPHALOMYELITIS IN WISCONSIN[1]

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The United States Bureau of Animal Industry began in the United States the collection of statistics on the incidence of equine encephalomyelitis in 1935. Every year since then, encephalomyelitis of horses has been diagnosed and reported in Wisconsin. The assumption that only the Western-type virus occurs in Wisconsin is not based on definitive evidence since the type of virus has never been identified. Veterinarians in the state, nevertheless, have used only the monovalent Western-type vaccine in horses. In the summer of 1952 we isolated Eastern equine encephalomyelitis virus from a horse which died in south central Wisconsin. Subsequently we demonstrated antibodies of the Eastern type in two other horses.

Experimental. In August of 1952 the Department of Veterinary Science, University of Wisconsin, examined, on behalf of the State Disease Control Laboratory, the brain of a horse (Horse A) forwarded by Dr. H. H. Bucholz of Wilton, Wisconsin. Confirmation of his diagnosis of equine encephalomyelitis was requested.

The allantoic chambers of 10 ten-day old embryonated hen eggs were inoculated with 0.1 ml. of a 10 per cent brain homogenate. The embryos were dead within 24 hours and all were contaminated with bacteria. In a second trial the brain homogenate was centrifuged at 3,000 r.p.m. for 20 minutes. The supernatant fluid was drawn off; sufficient penicillin and streptomycin were added to yield a final concentration of 2,500 units and 2,500 micrograms, respectively, per ml.; and 0.2 ml. was inoculated into the allantoic chamber of ten-day old embryonated eggs. One of the 10 embryos died, the death occurring 24 hours after inoculation. The embryo was hemorrhagic, the fluids clear and bacteria-free. The harvested allantoic fluid was inoculated into 6 eggs all of which died within 24 hours. No difficulty was experienced in propagating the agent in further passages.

Allantoic fluid harvested from the third egg passage, titrated in eggs, had an embryo LD_{50} of 10^6. Five-week old white mice inoculated intracerebrally with 0.05 ml. of virus developed ataxia, ruffled hair coats, scratching reflexes and tremors within 48 hours, and were dead within 60 hours. Specific St. Louis Eastern and Western encephalomyelitis antisera were obtained from the Communicable Disease Center, P.H.S., U. S. Department of Health, Welfare, and Education through the courtesy of Dr. Morris Schaeffer. Serum-neutralization tests in eggs showed the agent to be Eastern equine encephalomyelitis virus, the neutralization index being 790. The virus was not neutralized by St. Louis and

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Western encephalitis antisera. The virus was sent to Dr. Kissling of the Communicable Disease Center, who confirmed our findings (Kissling, 1952). Further evidence of the presence of the Eastern-type virus was obtained August 28, 1952, from a field investigation in the area from which the horse brain had been obtained two weeks earlier. Several horses with clinical encephalomyelitis had been reported by two local veterinarians but only three definite cases could be traced. Of these, two horses had died (Horses A and B) and a third had recovered (Horse C). Serum from this latter animal was found to possess complement-fixing antibodies for both Eastern and Western type antigens; fixation with the Western type antigen occurred in serum dilution of 1:64 and with the Eastern type in a dilution of 1:256. Later, Dr. Ralph Garman of Tomah sent serum from another clinically affected horse (Horse D). This serum also possessed complement-fixing antibodies against the Eastern type antigen, 1:64, and an insignificant titer, 1:4, against the Western type.

Chickens and ducks were bled and insects trapped on affected premises during the field investigation but all attempts to isolate the infectious agent from these species failed. While mosquitoes were not abundant on the day of the trip, possibly because of strong winds, the farmers maintained that they had been. The nature of the terrain, with numerous swamps and cranberry bogs, was an ideal breeding ground for mosquitoes.

Discussion. The demonstration of Eastern equine encephalomyelitis in an area where it was hitherto unsuspected suggested inadequacies in our knowledge of the natural history of the disease. All of the cases originated in a district about 36 square miles in area, having about 4 horses to a square mile, i.e., an attack rate of 36 per 1,000. Half of the clinical cases died. There were probably insufficient numbers of susceptibles for development of an epizootic such as occurred in southern Michigan in 1942 and 1943 where the number of cases totaled 102 and 367 respectively, with case mortality rates of 91 and 95 per cent (Miller, 1944; Mohler, 1943) respectively. The 1952 Wisconsin outbreak was detected only in horses. Inquiries failed to reveal any evidence of encephalitis in the human population, the farm families or the personnel of a large army base which was situated on the boundary of the affected area (Brown, 1952). The assumption that the Eastern type virus produces many fatalities in horses and must involve a large number of horses or men does not serve as a criterion for judging whether an outbreak is produced by Eastern or Western type virus or by any type of equine encephalomyelitis virus.

The demonstration of antibodies for both Eastern and Western equine encephalomyelitis in two sera suggests that the horses had been exposed to both viruses perhaps without developing clinical signs to one and that the complement-fixing antibodies to the first infection had persisted, and may have been enhanced by the second infection. Even though the horses were locally raised and had not been vaccinated, examination of sera from a greater number of horses and at several times of the year would be required to establish the fact that the antibodies to both encephalitic viruses resulted from infection. The Eastern virus was originally believed to occur only along the Atlantic seaboard.

Randall and Eichorn (1941) first showed the Eastern type to occur in Texas, and since then the virus has been found in Michigan (Mohler, 1943), Missouri (Miller, 1945), Louisiana (Oglesby, 1948) and Arkansas (Simms, 1950). Western equine encephalomyelitis has been found in four states east of the Mississippi: Alabama, Kentucky, Illinois, Michigan (Mohler, 1943).

SUMMARY

Eastern equine encephalomyelitis virus was isolated from a horse in south-central Wisconsin during August 1952. The disease was limited to a few horses in a small area of swampy country. Man was not involved.

Acknowledgement

The cooperation and assistance of Dr. H. H. Bucholz, Wilton, Wisconsin, and Dr. R. Garman, Tomah, Wisconsin is gratefully acknowledged. We are also indebted to Dr. R.. E Hall, State Disease Control Laboratory, and Dr. Lois Jones, University of Wisconsin.

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RABIES STREET VIRUS STRAINS IN THE SYRIAN HAMSTER

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Specific inclusion bodies present in the central nervous system of animals dying from rabies are called "Negri bodies" (Negri, 1903). These are most frequently demonstrated within, but are revealed occasionally outside the nerve cells. These inclusions are eosinophilic bodies, usually spherical, but also of other shapes, varying from 1 to 30 microns in size. The inner virus structure shows basophilic granules. "Negri bodies" in brains of hamsters infected with rabies virus are larger and more numerous than those in mice infected with rabies virus (Reagan *et al.*, 1952). For this reason the hamster was chosen for use in this experiment.

MATERIALS AND METHODS

The strains of rabies virus used in this study were obtained from Dr. A. N. Metcalf of the Pennsylvania Bureau of Animal Industry at Enola, Pennsylvania. These strains are as follows: 191247 (fox brain), 191427 (cow brain), 191248 (dog brain), 192510 (dog brain), 192486 (dog brain). These specimens had been proven positive for rabies at the Pennsylvania Bureau of Animal Industry, and were preserved in glycerin until initiation of the present study. The specimens were removed from the glycerin, ground in mortar with alundum, and diluted to 20% suspensions with physiological saline.

Each of ten 3-week-old Swiss albino mice was inoculated intracerebrally with 0.03 cc. of the above described virus from specimen 192486; the other 4 strains were inoculated likewise. Six to eight sick mice from group 192486 were killed; the brains were removed aseptically, examined and found to contain numerous "Negri bodies." These were pooled, ground in a mortar with alundum, diluted to a 20 per cent suspension with physiological saline and titrated intracerebrally in Swiss albino mice (3-week-old). Specimens 192510, 191248, 191427, and 191248 also contained numerous "Negri bodies," and suspensions and titrations were done in like manner. Specimen 192486 titrated 10^{-2}, 192510 titrated $10^{-2.5}$, 191248 titrated 10^{-3}, 191427 titrated $10^{-2.5}$, and 191247 titrated 10^{-3} intracerebrally in Swiss albino mice. These virus-bearing brain suspensions were used to intiate the present study.

Diagnosis of rabies in hamsters and mice was made on the following factors: (1) rabies symptoms present in hamsters, (2) demonstration of "Negri bodies" in the Ammon's horn of the hamster and mouse brains. The presence of Negri bodies was determined by staining touch preparations of the Ammon's horn with Seller's stain and examining the slides under an optical microscope.

Sixty healthy Syrian hamsters, 18 days old, were divided into 2 groups of 30 hamsters each. One group was designated as group 1 (rectal exposure) and the

TABLE 1

Response of hamsters exposed rectally to 5 rabies virus strains

VIRUS STRAINS	NUMBER ANIMALS EXPOSED	MIN TO MAX INCUBATION PERIOD, DAYS	NUMBER ANIMALS SHOWING SYMPTOMS	NEGRI BODIES
192486	6	5 to 7	6	Pos.
192510	6	5 to 6	6	Pos.
191248	6	5 to 6	6	Pos.
191427	6	6 to 8	6	Pos.
191247	6	5 to 6	6	Pos.

TABLE 2

Response of hamsters exposed intraocularly to 5 rabies virus strains

VIRUS STRAIN	NUMBER ANIMALS EXPOSED	MIN TO MAX INCUBATION PERIOD, DAYS	NUMBER ANIMALS SHOWING SYMPTOMS	NEGRI BODIES
192486	6	14 to 18	2	Pos.
192510	6	14 to 17	3	Pos.
191248	6	14 to 18	2	Pos.
191427	6	14 to 18	2	Pos.
191247	6	14 to 20	3	Pos.

Hamsters showing no symptoms were held 30 days and discarded.

other, group 2 (eye exposure). One-tenth cc. of a 20 per cent virus suspension from specimen 192486 was instilled rectally into 6 hamsters of group 1. The end of an 18-gauge needle was filed off, rough ends were smoothed down, and the tip was lubricated with vaseline before being inserted into the lumen of the rectum. 0.03 cc. of the same virus suspension was dropped into each eye of 6 hamsters from group 2. Specimens 192510, 191248, 191427, and 191247 were all done in like manner. The results are given in Table 1 and Table 2.

SUMMARY

Hamsters exposed rectally to 5 rabies virus strains showed an incubation period between 5 to 8 days in comparison to hamsters exposed intraoculary whose incubation period was between 14 to 20 days. Brains removed from hamsters showing rabies symptoms contained numerous "Negri bodies."

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A COMPARISON OF HELMINTHIC AND PROTOZOAN INFECTIONS IN TWO EGYPTIAN VILLAGES TWO YEARS AFTER THE INSTALLATION OF SANITARY IMPROVEMENTS IN ONE OF THEM

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Over a period of four years, from 1948 to 1951, the International Health Division of the Rockefeller Foundation, in cooperation with the Egyptian Ministry of Public Health, made an extensive study of health conditions in a group of five Egyptian villages which were believed to be representative of Egyptian rural villages in general (Weir *et al.*, 1952). This group of villages is situated in Calyubia Province, not far from Cairo. After a preliminary survey of health and sanitary conditions, certain sanitary improvements were installed and health services developed. These were completed by about the beginning of 1951, the final year of 1951 being devoted to maintenance and evaluation of the effects of the program on the basis of mortality statistics. In four of the villages varying numbers of installations and services were provided so that it might be possible to evaluate the services differentially, whereas one village, Aghour El Kubra, was left as a control, no sanitary changes having been made.

In the hope of obtaining further data on the effectiveness of the sanitary improvements provided in the foregoing project, after they had been in existence for about two years, the writer made a study of helminthic and protozoan infections in two of the villages, Sindbis and Aghour El Kubra. The latter, as noted above, had had no improvements provided, whereas Sindbis was provided with the following: 1) bored hole latrines topped by concrete platforms, for about 90 per cent of the houses; 2) wells providing an uncontaminated water supply, at the rate of one well for each 200 of population; 3) attempted fly control by application of insecticides to breeding areas, temporarily successful but ultimately a failure due to development of fly resistance to the chemicals used; 4) installation of a refuse disposal service; and 5) general preventive medical activities such as visiting nurses, venereal disease control and tuberculosis control. In addition, Sindbis had a Health Center established in 1948 to serve the entire area of five villages, but which naturally was much more extensively patronized by inhabitants of Sindbis than by those in the other villages. At this Health Center an effort was made to treat all cases of certain diseases which came to light during the preliminary survey, including hookworm, *Ascaris* and *Schistosoma* infections. Since the preliminary survey did not include the control village, Aghour El Kubra, this village did not benefit to any great extent from this extensive treatment program, whereas Sindbis did.

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A complete description of the villages studied will not be attempted since Egyptian villages are remarkably uniform and descriptions of them have been given in the American literature by Headlee (1933), Scott (1939) and others. However, certain pertinent information is necessary for an understanding of the situation. Sindbis has a population of about 4,200. Prior to the installation of sanitary improvements, the preliminary survey showed that 27 per cent of the houses in this village were provided with latrines, but these were of an unsanitary pit type which were prolific fly-breeding places and concentrated source of contamination for the flies, so that they were more of a hazard than a benefit. Wells were present in 17 per cent of the houses, but they were shallow, in close proximity to the latrines when these were present, and were universally contaminated to a high degree. Aghour El Kubra, with a population of about 9,000, is situated some four kilometers from Sindbis. In this village, 44 per cent of the houses had more or less unsanitary latrines, and 10 per cent had shallow wells.

Little difference in the general level of housing and sanitation, other than the improvements provided in Sindbis mentioned above, was apparent. As in all typical Egyptian villages, the mud houses, tightly clustered together, have dirt floors and poor ventilation. Usually there is an animal room, partly covered with litter, which is one of the commonest human defecation sites when there is no latrine, and often when there is. Not infrequently the flat roofs of the houses are also used as defecation sites; often they are used as poultry yards as well. Trash from either animal room or roof naturally finds its way to the floors of other parts of the house. Sometimes it is permitted to accumulate, but in other cases the floors of the rooms not utilized by animals are kept well swept. The litter from the animal room, including the animal dung and very often human stools as well, is usually collected daily, sometimes on alternate days, and carried to the fields or to community refuse piles in or adjoining the village. The litter is usually first swept into one or more piles and then placed by the hands in baskets. Some of the straw and manure is molded into fuel cakes by the hands of the women and children. Since, as Scott (1939) pointed out, Egyptian villagers live close to the ground, where they eat, sit, play and sleep, the opportunity for spread of intestinal infections from contaminated floors is obviously very great, especially since careful washing of the hands, even after gathering dung, or before eating, is not a common practice for adults any more than for children.

The installation of latrines and availability of pump wells does not necessarily mean that they are used. There has been much discussion about the extent to which the latrines are used. Little reliable information can be obtained by questioning. There is no doubt that the men and older boys who work in the fields much more commonly defecate in the fields than in the latrines in their houses, although they also use the latrines in the mosques when convenient for them to do so. It is also obvious from observation that the younger children and to some extent the adults, particularly women, who spend a fair part of the day at home, often prefer to use other sites than the latrines for defecation. This is due in part to the frequent failure of the latrines to be properly screened from other people in the house, and in part to their becoming soiled, smelly and repulsive

from careless use. We concluded that, at least during the period when we were making our observations, some of the latrines were probably not used at all, whereas others gave evidence of being used at least the greater part of the time. The children of school age made extensive use of the school latrines when these were available. At Sindbis the official school was provided with clean, well-kept latrines which, as far as we could judge, were used to the exclusion of any other sites, but the official school in Aghour El Kubra had less well-kept latrines. The "unofficial" schools in both places had no latrines, and vacant areas nearby were found polluted to an extreme degree.

As noted in the report by Weir *et al.* (1952), the pump wells installed in Sindbis were used to the exclusion of all other water supplies by people in the village, although people working in the field continued to drink water from the canals. In Aghour El Kubra some good wells were present, but many were polluted.

In order to study the protozoan and helminthic infections of Sindbis and Aghour El Kubra arrangements were made for the collection and examination of about 250 stool specimens from each of the two villages. No urine samples were collected for examination for *Schistosoma haematobium* infections, since with the time, facilities and assistance available, thoroughgoing examinations would have been difficult and would not, we believe, have given us much additional information on the benefits derived from the improvements in Sindbis. In the first place, egg counts are not a reliable method for judging the degree, or even the presence, of *Schistosoma* infections; second, the treatment given to most of the cases detected in Sindbis would have masked any falling off in reinfections; and third, most of the infections existing in 1950 would have still been present in 1952. Halawani (personal communication) has collected data indicating that *Schistosoma* infections tend to be focally acquired in places where pollution is concentrated, especially near villages, so at first sight the installation of latrines might be expected to reduce these infections in the course of time. However, children, who in Egypt have a very high infection incidence, and shed more eggs than do adults, continue to bathe and swim in the canals in and near the villages and to pollute them while doing so. It is doubtful, therefore, whether the latrines would have much effect on schistosomiasis even after a long time.

METHODS

Fresh stool samples were collected, as far as it was possible to obtain them in daytime visits, from inhabitants of the two villages, the visits alternating from one village to the other, on two days a week during November and December, 1952. Since it soon became obvious that complete collections from the residents of individual houses would be impossible by this method, no attempt was made to do this. The object of the survey was to compare the findings in one village with those in the other, and with previous findings in the same villages, rather than to compare house or families within the villages as Scott did in 1939. In a number of instances, however, three to five specimens were obtained from the residents of single houses.

House to house visits failed to produce a sufficient number of samples from

certain age and sex groups, e.g., adult males, who were almost all working in the fields, and children of school age, who were usually at school. Sufficient samples were obtained from the latter by visiting the schools, but we eventually had to resort to leaving sputum cups on one day for collection of early-morning specimens the next day, in order to get sufficient samples from adult males. Infants under one year of age were excluded from the study.

An effort was made to get from each village a minimum of 25 samples each from males and females in each of the following age groups: 1–4, 5–9, 10–19, 20–39, and 40 and above. This we succeeded in doing as far as examinations for helminth ova were concerned but, for reasons explained below, we did not get a full quota of samples for examination for Protozoa.

As soon as the stool specimens were obtained, two samples were collected, one for examination for helminths, the other for Protozoa. Approximately one gram of the specimen was measured into a 15 cc. screw-cap vial half filled with N/10 NaOH, using for measurement a spoon of 1 cc. capacity fitted into the cork of the vial by its stem. Another sample was placed with the aid of applicator sticks into a similar vial for examination by the MIF vial technic as described by Sapero and Lawless (1953). The vials into which the samples were measured contained 4.7 cc. of MF solution; just before addition of about 0.5 cc. of stool sample, 0.3 cc. of Lugol solution was added by means of a pipette calibrated at that point. The stool sample was then comminuted by means of the applicator sticks and shaking. As pointed out by Sapero and Lawless, it is important that the Lugol's solution be not more than three weeks old.

On two collection days an older sample of Lugol's solution was used by error and when microscopic examinations were made it became evident that the preparations were not as good as usual, and the usual number of trophozoites were not seen or were not specifically identifiable. These sets of specimens were, therefore, excluded from the protozoan examinations reported. At first we also excluded the samples collected from specimens left in the containers for several hours before we obtained them, but after discussion with Lawless, who had good success in finding cysts and flagellate trophozoites as well as some amebic trophozoites in such samples, and ourselves finding that the recorded findings were not materially different from those in fresh samples, we began to include these specimens in our survey report. Because of the exclusion of two sets of poorly preserved and stained samples, and some samples several hours old, our total number of protozoan examinations from Sindbis was 229, and from Aghour El Kubra 164, instead of the 250 or more in each place examined for helminths.

The examinations for helminth eggs were usually made on the day after collection, otherwise the specimens were kept in a refrigerator until examination on the second day. The specimens were thoroughly comminuted by means of applicator sticks, and the vial filled with N/10 NaOH to a 15 cc. mark to get a 1:15 dilution. Since the great majority of the stool samples were of mushy type, no record was kept of the consistency of each individual stool. Since the stools ran so nearly alike in the two villages, it was felt that an attempt to classify them by consistency, and alter the egg counts accordingly, might introduce more error

than it corrected. However, adjustments were made in the case of a few specimens, probably between 5 and 10 per cent, which were obviously either unusually dense or were partly or completely diarrheic. Only two stools of dysenteric type were encountered. In the case of thin, diarrheic specimens, either a smaller amount of fluid was added to the vial, or larger samples (1½ to 2 times) were counted. In the case of unusually dense specimens, obvious when samples were placed on a slide, smaller samples (¾ to ½ the usual amount) were counted.

No adjustments were made in the egg counts on the basis of stool size in young children, since the purpose of the present investigation was merely a comparison between two villages and was not concerned with the actual number of worms represented by the egg counts. Besides, as far as the worm burden of a community is concerned, the fact that a certain number of eggs per gram in a child's small stool represents a smaller total number of eggs passed per day, and therefore fewer worms than the same count in an adult's stool, is probably compensated for, so far as the seriousness of the helminthic infections to the community is concerned, by the fact that it takes fewer worms to cause a given amount of damage in a child than it does in an adult.

After preparation of the vials the contents were thoroughly shaken and a few cc. poured into aniother vial, from which a 0.075 cc. sample was withdrawn for egg counting. Duplicate counts were not made, since it was felt that more valuable data would be obtained by utilizing the time available in making a larger number of single counts than in making duplicate counts on a smaller number. The numbers of eggs per gram are given as 200 times the actual count. To bring the counts to the basis of formed stools, all of these counts should be multiplied by two (Augustine *et al.*, 1928).

The greater part of the fecal suspension in the original vial was used for flotation in saturated NaCl solution in order to detect light infections (with helminths having floatable eggs) not detectable by examination of $\frac{1}{200}$ cc. samples. With the exception of *Trichostrongylus* eggs, a few of which were present in a surprisingly large number of samples, only a few additional helminth infections, mostly hookworm and *Enterobius*, were detected by this method. In Table 1 the hookworm infections detected by flotation only are arbitrarily recorded as having 100 eggs per gram, although many probably had even fewer eggs.

SURVEY RESULTS

I. *Helminths*

The results of the helminthic surveys in the two villages are summarized in Table 1. The averages ("wtd av." in the table) are adjusted for the proportions of the inhabitants belonging to each age and sex group, as kindly provided by the Statistical Department of the Egyptian Ministry of Finance and Economics, based on the 1947 census. The population proportions in Sindbis were approximately as follows: Under 1 yr., 3%; 1–4, 11%; 5–9, 13%; 10–19, 18%; 20–39, 29%; 40 and over, 26%. The figures for Aghour were the same except that there were 19% in the 10–19 group and 28% in the 20–39 group. In the column for

TABLE 1

Helminth infections in Sindbis and Aghour El Kubra

AGE	SEX	ASCARIS				ANCYLOSTOMA		TSTR.	H. NANA
		No ex'd	% +	Av. epg + cases	Av. epg. all	% +	Av. epg + cases	% +	% +
Sindbis									
1–4	F	25	52	5400	2800	0	0	32	16
	M	24	54	3200	1800	12	140	16	16
5–9	F	25	62	4600	2400	4	100	37	16
	M	24	64	8200	4200	16	400	46	12
10–19	F	25	60	4400	2800	4	100	36	16
	M	25	40	5200	2000	12	140	20	20
20–39	F	42	62	5600	3600	19	540	29	5
	M	25	52	4600	2300	12	140	20	0
40+	F	27	37	3600	1800	0	0	26	0
	M	25	50	2600	1300	8	100	42	0
Wt'd Av.	F	144	51	4600	2660	7	187	30	Ages 1–19, 17
Wt'd Av.	M	122	48	3800	1900	12	144	28	Ages 20+, 1
Wt'd Av.	All	266	50	4200	2300	9.5	177	29	8
Aghour El Kubra									
1–4	F	25	68	5600	3000	8	200	44	4
	M	25	76	10600	8000	20	200	16	12
5–9	F	25	80	8400	6800	16	100	36	8
	M	25	84	9800	8200	40	260	32	12
10–19	F	25	80	5200	4200	12	160	28	4
	M	25	92	11600	10000	48	380	56	8
20–39	F	40	75	6000	4000	13	720	45	0
	M	25	80	7200	5700	52	540	44	0
40+	F	25	64	6400	4100	20	100	48	0
	M	25	64	4600	4700	44	240	44	0
Wt'd Av.	F	140	72	6200	4200	14	290	40	Ages 1–19, 8
Wt'd Av.	M	125	77	7600	6600	42	350	40	Ages 20+, 0
Wt'd Av.	All	265	76	6900	5450	28	320	40	3.5

OTHER INFECTIONS	NO. OF CASES SINDBIS	NO. OF CASES AGHOUR EL KUBRA
Enterobius vermicularis	9	11
Trichuris trichiura	0	4
Toxocara canis	0	1
Schistosoma mansoni	5	4
Schistosoma haematobium	1	2
Hymenolepis diminuta	1	9

Hymenolepis nana, instead of showing averages for the two sexes, which showed no significant difference, the averages are shown for the younger and older age groups, where a striking difference is apparent.

It will be seen that by far the most prevalent helminthic infection was *Ascaris*,

followed, as far as incidence was concerned, by *Trichostrongylus*, then *Ancylostoma*, and then *Hymenolepis nana*. Other infections, found in relatively small numbers, are listed at the foot of the table, with the number of cases found. Each infection will be discussed separately below.

Ascaris. The general incidence of this parasite, including all age and sex groups, was 50 per cent in Sindbis, and 76 per cent in Aghour El Kubra. In addition to this marked difference in incidence, the average epg. (eggs per gram) for the positive cases showed a similarly greater magnitude in Aghour El Kubra as compared with Sindbis—6,900 vs. 4,200. The average *Ascaris* burden for the entire populations, therefore, was nearly 2½ times greater in Aghour El Kubra than in Sindbis, i.e., 5,450 vs. 2,300 epg. According to the estimate made by Augustine *et al.* (1928) this represents an average of less than two pairs of worms per person in Sindbis, and about four pairs in Aghour El Kubra. In Sindbis 60 per cent of the positive cases had 3,000 epg. or less, whereas in Aghour El Kubra only 38 per cent fell in this category. Of 37 individuals (7 per cent) who had egg counts in excess of 15,000 per cc., 25 lived in Aghour, as did 10 of 14 whose epg. exceeded 25,000. Of particular significance is the fact that, if the figures for both sexes are combined, the average *Ascaris* burden in the infected individuals in the 1 to 4 age group is twice as great in Aghour El Kubra as in Sindbis.

Other than this difference in the 1–4 age group in the two villages, the small number of individuals examined in each age and sex group makes the differences in the *Ascaris* burdens which appear between individual age and sex groups in our study not statistically valid. Even when the figures for an entire village or both villages combined are considered, no statistically valid difference between the sexes is in evidence. Scott (1939) in his extensive survey of *Ascaris* infections in Egypt, stated that, as in many other countries, women have more infection than men, and children more than adults, although the differences are less than in some other countries. The incidence figures of Weir *et al.* (1952)—no egg counts were made—show the same thing. Our own figures are not more than suggestive with regard to age distribution, but as far as they go they are in agreement with Scott's findings that a maximum incidence and degree of infection develop before the 10th year. Our figures, however, show no indication of diminution in infection until after the 40th year, and even then the falling off is of moderate proportions. The largest number of counts over 15,000 (11) occurred in the 10–19 age group. The highest single counts were two of about 50,000, in a 4-year-old and 9-year-old child, respectively, both in Aghour El Kubra.

Besides the very marked difference in amount of *Ascaris* infection in the two villages, our incidence of 50 per cent in Sindbis in 1952, as compared with 62 per cent found by Weir *et al.* in their 1950 survey, is indicative of a true lowering of the amount of infection during the intervening time. The 1952 figures are lower in every age and sex group. No survey was made in Aghour El Kubra in 1950, but it is quite likely that the incidence then was not markedly different from that in 1952. In the other three villages in which surveys were made by Weir *et al.* the *Ascaris* incidences were 68.8, 84.4, and 87 per cent.

There is good reason to believe that the marked diminution in *Ascaris* infec-

tions in Sindbis was due to the sanitary improvements. It is true that while the 1950 survey was going on, a large proportion of the individuals found positive for *Ascaris* were given treatment at the Health Center. Otto and Cort (1934), however, have adduced convincing evidence, based on results obtained in four entirely different localities by different groups of workers, that in the absence of sanitary improvements a return to a pretreatment level of infection may be expected in from five to eight months. With the floors of the houses as heavily seeded with *Ascaris* eggs as they were found to be in the absence of sanitary improvements (see below), such a rate of reinfection would have been expected in Sindbis for a period of several months. This would probably have been enough to bring the *Ascaris* burden back to the pretreatment level where, had conditions remained as they were, it might have been expected to remain. However, the installation of the latrines and their use to a sufficient extent to decrease the contamination of the floors of the houses, as we showed actually happened, together with the fact that the usual life span of *Ascaris* is little more than a year, might be expected to cause a falling off in the *Ascaris* burden such as was observed, even if there was a rapid rate of reinfection at first. The reduction in the burden may have been greater than is indicated by the decline from 62 per cent to 50 per cent incidence, for the average burden in the infected individuals (4,200 epg.) is rather low. There are, however, no figures on individual burdens in 1950 for comparison.

Scott (1939) concluded that *Ascaris* infections in Egypt are not concentrated in certain families as they are in many places, but are fairly evenly scattered due to almost universal contamination of the ground in the compact, heavily polluted villages. He also concluded that the infection is transmitted primarily by direct hand-to-mouth transfer of eggs which have developed on the moist floors. Yet there is a very prevalent belief in Egypt that *Ascaris* infections are acquired to a large extent from eating vegetables grown in polluted soil and washed in polluted water.

Although we did not attempt to get specimens from all the members of very many families, there was some evidence that there was a considerable difference between some of the families, and that the pollution, though widespread, was not as uniform in these villages as Scott found in the places where he worked. To test this matter further we collected soil samples from various localities in Sindbis and Aghour El Kubra, and examined them for *Ascaris* eggs by the method described by Spindler (1929). We collected small specimens, only 25 gm. each, from the superficial layers of floors, latrine platforms, refuse piles and defecation areas, and from these, after thorough mixing, examined 5 gm. samples. No record was kept of the stage of development of the eggs, but almost all contained living embryos. The specimen that contained the largest number of eggs—28 in a 5 gm. sample—was collected by scraping earth from the sides of a latrine in the mosque in Aghour El Kubra. Samples from defecation sites near the "unofficial" schools in Sindbis and Aghour, where there were no latrines, all yielded eggs. Three samples from the playground of the official school in Aghour, where the latrines were in only fair condition, each yielded eggs (9, 6 and 4 per 5 gm. sam-

ple), whereas in samples from the yard of the official school in Sindbis, where the latrines were very clean and well cared for, no eggs were found. In Aghour El Kubra samples from the floor of a house from which four fecal specimens were obtained with 16,000, 13,000, 8,200 and 4,000 epg., respectively, yielded an average of 12 eggs each, whereas negative samples were obtained from an adjoining house with a well-swept, dry floor, from which one negative fecal sample was obtained, and one with a count of 2,400 in a schoolboy. Samples were taken from four houses in Sindbis where the egg counts in the residents from whom we obtained samples were: (1), 4 negatives; (2), 0,0,0 and 1,900; (3), 0,0 and 800; and (4), 10,600 and 11,400. In house (1) the soil samples from the floor were negative, although 2 eggs were found in scrapings from the platform of the latrine. In house (2) a sample from near the latrine and another from the moist floor near the water pump were negative. In house (3) one sample yielded a few eggs. In house (4) with the heavy infections, two samples were positive, one yielding 21 eggs.

Finding so many eggs in these small 5 gm. samples indicates a very high degree of pollution. Although the number of samples examined was far too small to warrant any definite conclusions, there seemed to be some correlation between the degree of infection in individual houses and the amount of contamination on the floors of these houses, suggesting some tendency towards family concentration as in other countries, though perhaps less marked.

In order to get some light on the comparative importance of vegetables as a source of *Ascaris* infections, we collected heads of several kinds of leafy vegetables which are usually eaten raw, and examined them for helminth eggs. Some of the specimens were pulled from the fields near Aghour El Kubra, where they had recently been watered from a canal; some were brought from a vendor in a street of this village; and some were obtained from the homes, where they were ready to be eaten, or were actually being eaten. Only the excess mud which the people wipe off before eating was removed from these specimens. All the leaves in a head of the larger leaf lettuce, or in several bunches of the smaller plants, were then individually washed and rinsed in 500 cc. or more of water, using a test-tube brush. The washings were allowed to settle overnight, the excess fluid siphoned off and the washings centrifuged. The latter were then processed for recovery of helminth eggs as were the soil samples described above. In spite of careful search of each specimen, no *Ascaris* or other helminth eggs were found. This result, together with the results of the soil examinations described above, corroborate the conclusions of both Headlee (1938) and Scott (1939) that hand-to-mouth transfer from contaminated soil in and around the habitations constitutes the primary source of *Ascaris* infections in Egypt.

Ancylostoma. As shown in Table 1, the general incidence of hookworm infection, including all age groups, was 7 per cent for females and 12 per cent for males in Sindbis, and 17 per cent for females and 42 per cent for males in Aghour El Kubra, but in both villages the average egg count in the positive cases was extremely low, averaging, on the basis of the mushy stools examined, less than 200 in Sindbis and little over 300 in Aghour El Kubra. In both villages approxi-

mately one-half of the positive cases were so light that they were found only by flotation, and the 100 epg. arbitrarily used for these in getting the averages in the table was probably too high. In Sindbis only 2 individuals, both in the 20–39 age group, had over 1,000 or more epg., the highest count being 1,600. In Aghour El Kubra, 4 individuals had over 1,000 epg., all of them between the ages of 19 and 30; the highest count was 2,400.

It is obvious that in spite of the unexpectedly high incidence of hookworm infection for a village in Lower Egypt which we found in Aghour El Kubra—several times higher than any of the figures reported by Weir *et al.* for this group of villages—the infections are so light (average of about two pairs of hookworms per person) that, even with the rather poor nutritional status in these villages, ancylostomiasis may be considered of negligible public health importance.

All of the individuals with 1,000 or more epg. were young adults who worked in the fields. It seems evident that only very light hookworm infections are acquired in the villages themselves. In Sindbis only two individuals under five years of age were found infected, both so lightly that they were detected only by flotation. In Aghour El Kubra there were 7 infections in the 50 children under five who were examined; one was detected only by flotation; in five, only one egg was found on a slide, indicating about 200 epg.; and in one, 2 eggs were found on a slide. It would seem, therefore, as was found to be the case with *Ascaris*, that there is evidence of more exposure to hookworm infection in Aghour El Kubra than in Sindbis. It should be noted, however, that we found a 7 per cent incidence of infection in Sindbis, whereas Weir *et al.* found only 3.5 per cent. This may have been due in part to the time of the year when the examinations were made, and perhaps in part to differences in technic.

Trichostrongylus spp. The incidence of light *Trichostrongylus* infections was remarkably high in both villages, the general average being 29 per cent in Sindbis and 40 per cent in Aghour El Kubra. As will be seen from Table 1, there was no clear evidence of differences with respect to age or sex. In the great majority of cases the eggs were detected only by flotation, and were often so scanty even by this method that the possibility of their having been swallowed must be considered. Repeated examinations of considerable numbers would have been necessary to determine this point, but this was not done. The highest epg. found for *Trichostrongylus* was 600. No significance from a health standpoint can be attached to these light *Trichostrongylus* infections, but they reflect the close association with animals in the homes.

Enterobius vermicularis. As might be expected from fecal examinations, only a few pinworm infections came to light, 9 in Sindbis and 11 in Aghour El Kubra. Contrary to what might have been expected, those that were found were fairly evenly distributed in all the age groups.

Trichuris trichiura. Whipworm eggs were found in only four individuals, all in Aghour El Kubra. This is not unexpected where desiccation outside the body of a host is as great a hazard as it is in this part of Egypt. *Trichuris* is more susceptible to injury from desiccation than is either *Ascaris* or *Ancylostoma*. *Ascaris*

eggs are much more resistant to drying, and hookworms have to survive in the egg stage for only a short time, after which the free larvae, to some degree and in certain circumstances are able to protect themselves from desiccation.

Schistosoma spp. We found only 9 cases (1.7 per cent) of *S. mansoni* in examination of $\frac{1}{200}$ gm. of feces; 5 were found in Sindbis and 4 in Aghour El Kubra. We also found eggs of *S. haematobium* in one fecal specimen in Sindbis and two in Aghour El Kubra. It is clear that this represents only a small fraction of the existing cases, since Weir *et al.* (1952), examining the stools by several methods, found 21 per cent of the males and 5 per cent of the females in Sindbis harboring *S. mansoni*. These authors reported that by using refined and accurate diagnostic methods, 95 per cent of 215 males in Sindbis were found to harbor *S. haematobium*.

Hymenolepis nana. This tapeworm was found in 36 cases (7 per cent), 25 in Sindbis and 11 in Aghour El Kubra. The fact that it was not, like *Ascaris*, more prevalent in the latter village where there is a higher degree of human fecal pollution, corroborates the writer's opinion (Chandler, 1949) that human infections with this worm are most frequently acquired by contamination of food with the droppings of rats or mice. These rodents are common in the villages studied and ample opportunity was afforded for contamination of food.

This is the only helminth infection in which a very striking difference in rates of infection was observed in different age groups. Of the 36 cases, all but two occurred in individuals under 16 years of age. It is improbable that the older individuals are much more careful about eating food contaminated by rodent droppings than are the younger ones, so changes in habits leading to decreased exposure is presumably not the explanation. It is also unlikely that it is due to development of specific immunity, since this would not be expected to develop so uniformly at a certain age. The loss of infections therefore appears to be a true age immunity, and to be associated with the attaining of puberty. Since a nearly related tapeworm, *Hymenolepis diminuta*, has been shown to be influenced by the host's sex hormones (Addis, 1946; Beck, 1951), it is possible that the sex hormones may play some part in this phenomenon, although in the case of *H. diminuta* in its normal host, it is the *absence* of the sex hormones which is inimical to the worms. A comparable change in *Giardia* infection at about the time of puberty was observed (see below).

Hymenolepis diminuta. Ten cases of infection with this parasite were discovered, nine in Aghour El Kubra and one in Sindbis. Since rodents and grain beetles have easy access to stored bread, cheese, etc., in both villages, we could find no reason for this difference between the findings in the two villages and think it may be due to chance. Unlike the *H. nana* infections, these *H. diminuta* infections were fairly evenly distributed in all age groups.

Other helminths. A single egg of *Toxocara canis* was found in an adult female. It is quite possible that this egg was swallowed and did not represent a true infection. No cases of *Strongyloides* or *Taenia* infections were found, nor were eggs of any flukes other than *Schistosoma* observed in our series of examinations.

II. *Protozoa*

The results of the examinations for Protozoa in the two villages are summarized in Table 2. These examinations were made by the writer at the U. S. Naval Medical Unit No. 3 (NAMRU 3) using facilities kindly provided there. Mr. D. K. Lawless, coauthor of the MIF technique, kindly helped with specific identifications when these were in doubt. Examination of each specimen was limited to a 10-minute search, using a 4 mm. objective and a 6× eye piece, specific diagnoses being made under oil immersion when necessary. Undoubtedly more protozoan infections would have been discovered if a longer time had been devoted to searching each specimen, and many more if repeated examinations had been made on separate days. As reported in part by Weir *et al.* (1952) and by Lawless in a personal communication, in an intensive search of 98 individuals, excluding babies, from Aghour El Kubra and 80 from another village, 96 per cent and 97 per cent were found to harbor *Endamoeba histolytica* when six separate stool samples were examined. Similarly high incidences of *E. coli* and *Endolimax nana* were found. In other words, practically, if not actually, 100 per cent of the older children and adults harbor *E. histolytica*. Our figures, therefore, of 55 and 59 per cent incidences of this parasite probably represent no more than 60 per cent of the existing infections, and this is probably true for our percentages for the other species of amebae also. For the flagellates the discrepancies between our records and the number of actual infections are probably even greater, since these Protozoa tend to appear and disappear, in numbers sufficient for easy detection, even more than do the amebae. Because of their small size, and the writer's lack of experience in recognizing them, no cases of *Enteromonas hominis* were recorded, although Lawless (personal communication) has found this to be a common parasite in Egyptian villages. Regardless of these deficiencies in our records, we believe that our figures on protozoan incidences in the two villages give a fair basis of comparison of them.

Since in no instance was there any statistically valid difference between the sexes in the incidences of protozoan infections, the figures for the two sexes are combined in each age group. It will be seen at once that the general incidences of infection are strikingly similar in both of the villages for all eight of the species of Protozoa found. This similarity was corroborated by an analysis in each of the villages of the number of individual species harbored by the inhabitants. As can be seen from Table 3, the similarity between the two villages is remarkable; the average number of separate infections for the entire populations is 2.3 species in each case, and the proportions with 1 species, 2 species, etc. is remarkably, alike in both villages. Combining the data for both villages, it will be seen that there are more negatives and single infections in the 1–4 age group than in any of the older groups, the average per person being 1.8. In all the older groups the average number of infections remains remarkably constant, from 2.3 to 2.5.

It is evident from these data that the sanitary improvements in Sindbis had not yet been reflected in a reduction in the incidence of protozoan infections. This is not surprising, since there is no evidence to lead one to expect any appreciable number of protozoan infections existing in 1950 to have disappeared by 1952.

TABLE 2

Incidences of protozoan infections in percentages

AGE	% OF POPULATION	NO. EXAMINED	*E. coli*	*E. histolytica*	*Endolimax*	*Iodamoeba*	*Dientamoeba*	*Giardia*	*Chilomastix*	*Trichomonas hominis*
Sindbis										
1–4	11	37	46	51	44	27	19	16	8	1 case
5–9	13	44	50	45	39	45	9	27	7	
10–19	18	42	60	48	57	29	14	16	2	
20–39	29	64	67	62	48	35	9	5	15	1 case
40+	26	42	69	60	55	38	14	2	14	2 cases
All	*97	229	58	55	49	35	12	**11	10	1.8
Aghour El Kubra										
1–4	11	31	32	32	32	22	3	16	9	1 case
5–9	13	38	60	58	40	40	16	16	10	
10–19	19	40	65	55	55	43	8	10	13	
20–39	28	40	60	71	57	40	20	6	9	
40+	26	20	55	60	50	45	5	0	10	1 case
All	97	169	56	59	50	40	11	**7.5	10	1.2
Both Villages Combined										
1–4	11	68	40	43	34	25	12	16	9	
5–9	13	82	56	52	40	43	12	22	8	
10–19	19	82	62	51	56	36	11	13	7	
20–39	28	104	65	66	52	37	13	5	13	
40+	26	62	65	60	52	40	7	1	13	
All	97	398	60	57	49	37	11	9	10	
1–9	24	150	48	48	37	37	12	**16	8	
10+	73	248	64	60	53	38	10	**3	13	

* The 3% under 1 yr. of age were not included. The "all" percentages are adjusted to the proportions of the population in each age group.

** For ages 1–19, 19% in Sindbis and 13% in Aghour; for ages above 19, 4% in Sindbis and 3% in Aghour; in combined table, for Giardia alone, the split in ages is at 19 yrs., instead of 9 yrs.

Since there is clearly no difference in the general incidences of infection in the two villages the figures from both may be combined to give a better basis for comparison of the amount of infection with the various protozoan species in the various age and sex groups, It will be seen that for all of the four commoner amebae—*Endamoeba coli*, *E. histolytica*, *Endolimax*, and *Iodamoeba*—the incidences found have a tendency to become progressively greater with advancing age. A comparison of the incidences in ages 1 to 9 as compared with those in older ages is shown at the bottom of Table 2. These differences may, however, be due to the fact that the parasites were more consistently present in recognizable numbers in the older individuals, rather than to an actual difference in incidence. The figures for *Dientamoeba fragilis*, *Chilomastix mesnili* and *Tricho-*

monas hominis are too small for comparison of the different age groups. *Giardia lamblia*, however, shows the same striking change in incidence in the age groups from 1 to 19 as compared with those above 19 as was shown by *Hymenolepis nana*. As for that parasite, reduced exposure to infection or development of specific immunity does not appear to be adequate explanation, and age immunity, possibly connected with changes at puberty, seems to be suggested.

SUMMARY AND CONCLUSIONS

Comparative surveys were made of helminth and protozoan infections in two Egyptian villages situated near together. One, Sindbis, had had bored hole latrines installed in the majority of the houses, and had had an unpolluted water supply made available for the entire population two years previously, while the other, Aghour El Kubra, had been left as it was.

1. In Aghour El Kubra the *Ascaris* incidence was 76 per cent and the average eggs per gram in the infected individuals 6,900, whereas in Sindbis the corresponding figures were 50 per cent and 4,200. The incidence in Sindbis in 1950 was 62 per cent. We believe these figures to indicate a definite lowering of exposure to *Ascaris* infection in Sindbis as a result of the sanitary improvements.

Search for *Ascaris* eggs in soil samples and washings from vegetables confirmed that the dirt floors of the houses are a more important source of *Ascaris* infections than uncooked vegetables. There was also evidence of correlation between the degree of infection in members of an individual household and the amount of contamination on the floors of these houses, indicating some tendency towards family concentration of *Ascaris* infections.

2. In Aghour El Kubra the *Ancylostoma* incidence was 17 per cent for females and 42 per cent for males, whereas in Sindbis it was 7 per cent for females and 12 per cent for males, but in both villages the individual burdens were very low. The data on age and sex infections suggest that a certain number of very light hookworm infections are acquired in the villages themselves, and that there was more exposure to such infection in Aghour El Kubra than in Sindbis.

3. Light *Trichostrongylus* infections were very common, 29 per cent in Sindbis and 40 per cent in Aghour El Kubra, reflecting the close association with animals in the homes.

4. *Trichuris* infections were few, again confirming the exceptional susceptibility of this parasite to relatively dry environmental conditions.

5. *Hymenolepis nana* was found in 7 per cent of the people examined, and about twice as frequently in Sindbis as in Aghour el Kubra. This again supports the view that this parasite is commonly acquired from food contaminated by rodent droppings, since otherwise a higher incidence would have been expected in the village with poorer sanitation. All but 2 of the 36 infections were in individuals below the age of puberty. *Hymenolepis diminuta* was found in 9 cases, but unlike the *H. nana* cases, these were evenly distributed in all age groups.

6. Special techniques for detection of *Enterobius* and *Schistosoma* infections were not employed, so only a few of these were found. No other fluke eggs or *Taenia* eggs were observed.

TABLE 3

Incidence of single and multiple protozoan infections in Sindbis and Aghour El Kubra, and in various age groups

	NO. EX'D	NEG %	1 SP. %	2 SP. %	3 SP. %	4 SP. %	5 SP. %	6 SP. %	7 SP. %	AV. NO. SP.
Sindbis	229	9	23	24	24	12	5	2	0.4	2.3
Aghour	164	11	22	21	26	13	6	0.6	0	2.3
Age 1–4	68	24	30	16	12	10	9	0	0	1.8
" 5–9	82	10	21	27	22	16	5	0	0	2.3
" 10–19	82	7	20	26	32	10	4	1	0	2.4
" 20–39	99	6	22	18	30	16	6	0	1	2.5
" 40+	62	5	21	29	28	8	7	3	0	2.4

No difference in the incidence of the various species of Protozoa was found between the two villages, and the average number of different infections harbored per person (2.3) was identical in both places. This number was lower (1.8) in the 1–4 age group, but was fairly uniform (2.4 to 2.5) in all the higher age groups. The failure of the sanitary improvements in Sindbis to be reflected in a lower incidence of protozoan infections may be due to loss of very few of the infections that were in existence two years previously.

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PROBLEMS IN TROPICAL PUBLIC HEALTH AMONG WORKERS AT A JUTE MILL NEAR CALCUTTA

I. Malaria in the Labor Population

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While agricultural pursuits still claim the vast bulk of India's labor force, increasing emphasis is being placed on the development, expansion and efficient operation of industries of many types. Industrial concerns in India, whether of indigenous or foreign origin, are faced not only with problems arising from purely occupational or environmental hazards, but must also somehow cope with the even greater problems involved in the maintenance of a suitable state of general health among their workers. As in most other underdeveloped countries, local and national health facilities are very limited and disease is so widespread that the health burdens fall largely on the individual enterprise, at least insofar as it may wish to ensure a given level of production efficiency and reduce its labor turnover.

Among the most economically important and extensively developed industries in India is the manufacture of jute products, and almost all of this industry is concentrated in the hundred-odd mills that line either side of the Hooghly (Ganges) River north and south of Calcutta. It was at one such mill, that of the Ludlow Jute Co., Ltd., an American concern with headquarters in Boston, that an opportunity was afforded during 1951–1952 to undertake a series of studies directed at assessing the relative importance of the various parasitic infections prevalent in the labor population. This first report deals with a year-long study of malaria; subsequent papers in the series will be concerned with intestinal parasitism, filariasis, and hematological observations among Ludlow workers.

THE LOCALE, WEATHER, AND PEOPLE

The mill compound of the Ludlow Jute Company is located on the left bank of the Hooghly River at Chengail, Howrah District, 17 rail-miles south of Calcutta and some 65 miles north of the outlet of the river into the Bay of Bengal (Fig. 1). The compound is about one mile in length and a third of a mile in depth, bounded on one side by the tracks of the Bengal-Nagpur Railway and on the other by the river itself. The Hooghly is navigable by ocean-going vessels as far north as the port of Calcutta and, despite the 80 miles that lie between Calcutta and the coast, it is subject to very strong tidal influences that produce a twice-daily rise and fall of as much as 10–14 feet. Because of these penetrating tides the river is brackish, the degree of salinity increasing during the dry periods of the year and decreasing with the onset of the rains. Since most of the countryside lies at or below the level of mean high tide, the river is embanked along most of its length and only small drainage channels and lock-controlled canals interrupt these "bunds." The land is flat, deltaic in character and alluvial in origin, and apart from the industrial establishments on the riverbanks the community

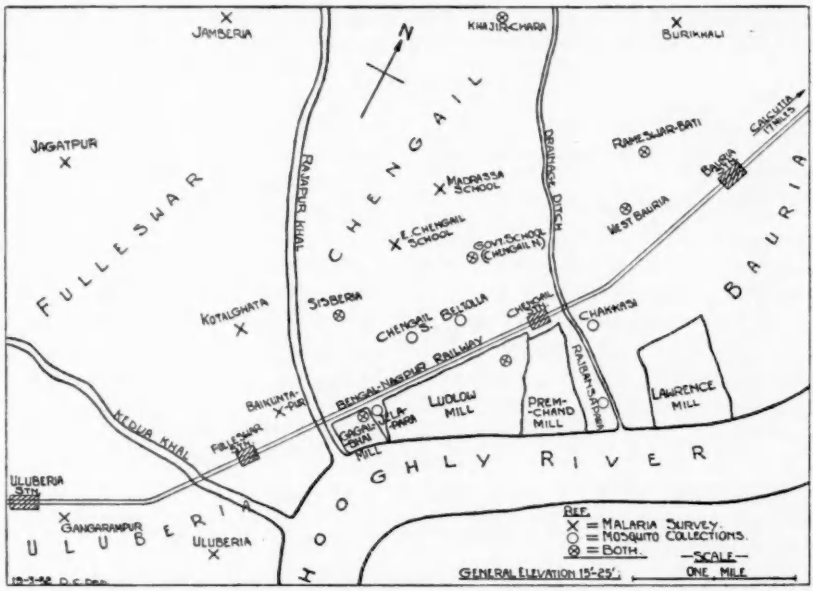

FIG. 1. Sketch map of the area surrounding the Ludlow Jute Company mill at Chengail, Howrah District, West Bengal. Locales where spleen and blood smear examinations were conducted and where routine mosquito catches were made are indicated.

is an entirely rural one and wholly typical of the Bengal countryside. Paddy fields dominate a landscape which is otherwise broken only by groves of scrub growth and palms, small elevated footpaths and narrow roads, and by the clusters of houses and sheds that are the local villages. Weather data compiled at Ludlow during 1951–1952 are summarized in Table 1.

The Ludlow mill at Chengail (Fig. 2) employs 6,000–7,000 workers in the processing activities that serve to convert raw jute into such widely used manufactured articles as bagging, sacking, webbing and hessian. Approximately half of the workers are of local Bengali origin and live in villages surrounding the mill at distances of up to eight miles. The other half of the working force is composed of men and women emigrated from Madras, Bihar, Orissa and other states. These individuals are for the most part housed within the Ludlow compound in blocks of buildings ("lines") built and owned by the company and rented at nominal cost. Most of these workers are not accompanied by their families, but sufficient dependents are maintained in the lines to bring its population up to an estimated 4,500 persons. Each rectangular barrack is divided into a series of stall-like rooms (in two rows of ten, set back-to-back); the rooms measure 9′ x 9′ (exclusive of a front verandah measuring 9′ x 4′) and may be occupied by 2 to 9 persons. The floors are of concrete and the walls of burnt brick, plastered and washed with lime; a small window and a door represent the only sources of natural illumination and ventilation in each room. Streets

TABLE 1

Weather data compiled at the Ludlow mill, Chengail, 1951–1952

MONTHS 1951–52	TEMPERATURE (°F.)				DAYTIME AVERAGE*	AVERAGE† DAYTIME RELATIVE HUMIDITY (%)	TOTAL RAINFALL (INCHES)
	Maximum		Minimum				
	Absolute	Mean	Absolute	Mean			
Aug.	95.0	89.9	75.0	82.2	85.5	—	13.40
Sept.	96.0	89.8	75.0	81.4	86.0	83.5‡	8.58
Oct.	95.0	89.5	74.5	79.0	84.3	81.1	4.36
Nov.	90.0	85.2	60.0	68.5	78.1	70.6	1.69
Dec.	87.5	81.2	56.0	60.4	71.9	66.0	0.00
Jan.	87.0	81.9	54.0	58.7	71.5	63.7	0.00
Feb.	97.0	89.2	59.0	65.6	78.2	58.7	0.00
Mar.	98.0	90.1	63.0	71.5	81.5	62.9	0.93
Apr.	105.0	94.8	70.0	77.9	86.5	70.3	4.83
May	98.0	93.4	69.0	80.2	87.5	73.3	3.36
Jun.	102.0	94.4	75.0	80.8	87.4	75.5	5.66
Jul.	95.0	87.9	75.0	80.0	83.7	84.4	27.52
Totals	105.0	88.9	54.0	73.9	81.8	71.8	70.33

* Average of temperature readings recorded daily at 8 A.M., 12 Noon, 5 P.M. and 10 P.M.

† Average of relative humidities recorded daily at 8 A.M., 12 Noon and 5 P.M.

‡ Partial record representing the last half of September. No records are available for August.

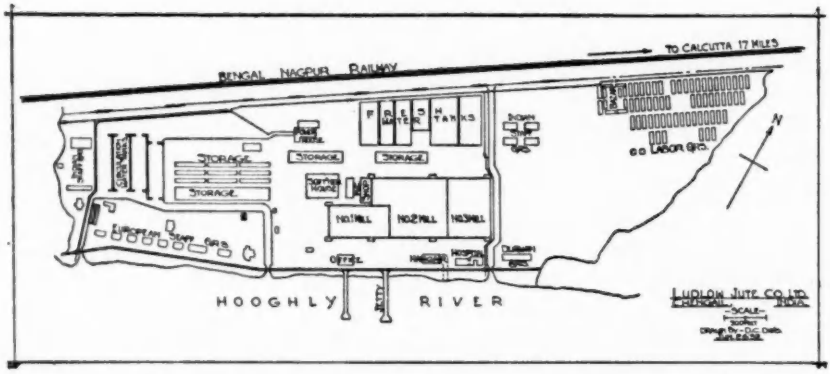

FIG. 2. Sketch plan of the Ludlow compound at Chengail showing its general organization and the disposition of living quarters.

are paved with brick and provided with surface drains, and the area is cared for by a group of sweepers under the direction of the medical department. Filtered, chlorinated water is supplied for cooking and drinking purposes through outdoor hydrants. A series of sanitary septic tanks together with a sewage disposal system serve for the disposition of excrement. Quarters in the labor lines are much sought after, for, inadequate as they may appear, they are much supe-

rior to village facilities. The villagers live in small huts made of bamboo, mud, cowdung and thatch, have no sanitary arrangements, and draw their water from communal ponds or "tanks" which also serve for bathing and laundering purposes and for the watering of stock. During the rainy season, when ditches, paddy fields, tanks and borrow pits fill to overflowing and more water is in evidence than land, sanitation becomes even more complicated and movement by foot the only sure way of negotiating the back-country paths.

The "European" supervisory staff of the mill is composed of persons of American, English, Irish, Scotch and Indian origins, who, together with their families, number 60-odd in all. Every effort is made to maintain a high level of good health among this group, and such provisions as distilled drinking water, screened windows, air-conditioned bedrooms, and social and sports facilities are available to all who live there. Also available to staff as well as workers, are the facilities of a new hospital-dispensary staffed by three full-time medical officers and provided with 18 beds, a dispensary, pharmacy, complete X-ray and clinical laboratory equipment, and an air-conditioned operating theatre.

The adjacent mills, Gagalbhai on the west and Premchand on the east, are similar to Ludlow in general organization although their medical facilities are limited to a single physician and a small dispensary and their workers quarters are older and less well maintained. The Gagalbhai environment was selected as a reference point for comparison with Ludlow because of its proximity and because, unlike the Ludlow labor and staff quarters which have been DDT-sprayed thrice yearly since 1948, the dwellings at Gagalbhai had never been so protected. The Gagalbhai labor force totalled 2,300 and its composition and local distribution resembled that described for the Ludlow employees.

PREVIOUS INVESTIGATIONS AND HISTORY OF MALARIA

The area of Howrah District along the Hooghly River was for many years considered healthy in terms of its low malaria incidence, and it is so now by comparison with many other areas in India. In October and November, 1930, however, malaria broke out in epidemic form in the resident population of a concentration of jute mills at Budge-Budge, up-river from Chengail. In these mills and their immediate vicinity spleen indices between 78 and 100 per cent were recorded and 50 per cent of blood smears taken were malaria-positive, the majority with *Plasmodium falciparum* (Iyengar, 1931). A search of houses revealed high densities of mosquitoes with *Anopheles sundaicus* (= *ludlowi*) predominating to the extent that the species exceeded "in number all other local species of anophelines many times over." A natural infection rate of 23.4 per cent resulted from the dissection of over 800 *A. sundaicus* taken from these mill areas, and Iyengar concluded that "both from the point of view of numerical prevalence and from that of susceptibility to natural infection, *A. sundaicus* is pre-eminently the important mosquito carrier in the area." In the Chengail area proper, Iyengar found spleen indices of 18 to 73 per cent in the villages just west and north of the Premchand mill (see Fig. 1), although the index in the Ludlow labor lines was only 8 per cent and those further to the east even lower. Brackish water ponds and ditches near Rajbansapara were breeding *A. sundaicus* in profusion

in close proximity to the villages with the highest spleen indices; a natural infection rate of 4.7 per cent was established for *A. sundaicus* taken from this area.

A. sundaicus is a brackish water breeder that was confined for many years to the mangrove swamp areas of the Sunderbans on the Bengal coast. From the coastal belt the mosquito has, over the years, steadily moved inland, probably through the agency of the railways and country-boats (Iyengar, *loc. cit.*), and has established itself in pockets near tidal channels and other bodies of brackish water where proper conditions of salinity and vegetation provide a suitable habitat in which protection is afforded against tidal wash. Adhikari and Ganguli (1949), working in Howrah District during 1945–1947, found oocyst rates of 0.0 to 1.5 per cent and sporozoite rates ranging from 0.0 to 2.2 per cent in *A. sundaicus*; infected specimens were taken only during the months of October and November. Sen (1948) reported a 10 per cent infection rate from a small number of specimens dissected at Fuleswar in 1933 and in general found that the incidence of natural infections in *A. sundaicus* in Bengal reached a peak in August and September.

Dispensary records at the Ludlow mill indicate that the most recent marked increase in malaria incidence occurred in 1945 when, during October and November, malaria rates were of the order of 250 cases per 1,000 workers. Since that time the autumn and winter rates have fluctuated far below that figure and malaria has not reappeared in the serious epidemic form it took throughout the area in 1930.

CURRENT STUDIES

Spleen and blood examinations

The first spleen and blood survey was conducted from August 17 to September 17, 1951, during the period immediately preceding the anticipated annual increase in malaria incidence characteristic of October and November. The examinations were conducted at 14 primary schools in the area surrounding the mill (Fig. 1) and in the Ludlow and Gagalbhai labor lines among the children resident therein. The results of the 1,104 spleen and 230 blood smear (thick and thin film) examinations are summarized in Tables 2 and 3 and indicated a uniformly low general incidence of malaria, quite in keeping with the preseason timing of the survey.

The second survey was planned for execution just after the peak of the seasonal rise in malaria rates (as judged by Ludlow dispensary figures) but a comparable sample of children could not be secured at this time because of the occurrence of an annual school holiday coincident with the harvesting season. This survey, conducted from November 28 to December 8, 1951, involved 316 spleen examinations and 248 blood smears, and the results (Tables 2 and 3) were indicative of only a slight rise in the general incidence of malaria in the area as a whole. One series of results, however, were in marked contrast to the others: among children in the Gagalbhai lines the spleen index had risen from 3.6 per cent to nearly 15 per cent and the prevalence of detectable parasitemias had markedly increased from 0.0 to 58.1 per cent. Thus, an intense but focal epidemic of malaria was in progress in the Gagalbhai lines, in very sharp contrast

TABLE 2

The results of 2 routine spleen and blood smear surveys conducted among children at and near Ludlow between August 17–September 17, 1951 (upper portion), and between November 28–December 8, 1951 (lower portion)

TOWN OR AREA	NUMBER OF CHILDREN		SPLEEN INDEX (PER CENT)	NUMBER OF BLOOD SMEARS		PARASITE INDEX (PER CENT)
	Examined	Enlarged spleen		Prepared	Positive	
Ludlow Lines	202	7	3.5	80	0	0.0
Gagalbhai Lines	56	2	3.6	16	0	0.0
Gangarampur	76	1	1.3	—	—	—
Chengail Govt.	75	0	0.0	19	0	0.0
Chengail East	70	2	2.9	—	—	—
Chengail Madrassa	66	2	3.0	30	0	0.0
Kotalghata	57	0	0.0	—	—	—
Baikuntapur	57	0	0.0	—	—	—
Jagatpur	53	0	0.0	21	0	0.0
Khajir-Chara	60	2	3.3	—	—	—
Jamberia	57	0	0.0	28	0	0.0
West Bauria	31	0	0.0	14	0	0.0
Uluberia	60	1	1.7	—	—	—
Bhurikhali	100	3	3.0	—	—	—
Rameswar-Bati	24	0	0.0	—	—	—
Sisberia	60	0	0.0	22	0	0.0
Totals	1104	20	1.8	230	0	0.0
Ludlow Lines	83	3	3.6	76	1	1.3
Gagalbhai Lines	47	7	14.9	31	18	58.1
Sisberia	52	1	1.9	52	3	5.8
Chengail Madrassa	50	2	4.0	41	1	2.4
Chengail East	49	2	4.1	48	0	0.0
Chengail Govt.	35	0	0.0	—	—	—
Totals	316	15	4.7	248	23	9.3

to the prevailing conditions elsewhere. Of the 23 positive blood smears derived from this survey, 17 (74 per cent) were *P. vivax*, 4 (17 per cent) were *P. falciparum*, and 2 (9 per cent) were mixed infections; quartan malaria was not in evidence.

ENTOMOLOGICAL STUDIES

General. Regularly scheduled mosquito catches were conducted throughout the entire year in the buildings of the Ludlow and Gagalbhai lines and in 10 surrounding villages (see Fig. 1) by 2 trained assistants. During a given week 6 of the 12 collecting areas were visited on successive mornings (6:30–9:00 A.M.) and the remainder during the afternoons (2:00–4:30 P.M.); the catching hours were reversed each week so that the monthly sample for any one area was ordinarily a product of 2 morning and 2 afternoon catches. Routine catches were conducted in human habitations as well as in cattlesheds and, so far as practica-

TABLE 3

Age distribution of spleen categories as determined by combined data from all areas surveyed August 17–September 17, 1951 (upper portion), and all areas surveyed November 28–December 8, 1951 (lower portion)

SPLEEN CLASS (HACKETT)	AGE GROUP				TOTAL
	0–1	2–4	5–9	10–14	
0	12	71	574	427	1084
1	0	1	10	7	18
2	0	0	1	0	1
3	0	0	0	1	1
4	0	0	0	0	0
5	0	0	0	0	0
Totals	12	72	585	435	1104
0	2	26	200	73	301
1	0	2	6	2	10
2	0	0	2	2	4
3	0	0	1	0	1
4	0	0	0	0	0
5	0	0	0	0	0
Totals	2	28	209	77	316

ble, the same buildings were searched at each visit to a given locale. In routine operation, an examination of a dwelling or shed involved a standard 15 minute search of all accessible surfaces from the floor level upwards. Captured mosquitoes were returned to the laboratory for immediate identification and for the segregation of specimens to be retained for dissection. During the period from August 1951 through July 1952, collections were made from a total of 2,032 dwelling units and 2,064 cattlesheds; these yielded 7,439 and 31,323 female anophelines respectively, a total of 38,762 for the year's activities. The anopheline fauna of the region includes 13 species which are listed below in order of their prevalence:

SPECIES	PER CENT OF TOTAL CATCH
A. subpictus Grassi	31.1
A. barbirostris Van der Wulp	18.1
A. annularis Van der Wulp	15.5
A. vagus Dönitz	13.6
A. hyrcanus nigerrimus Giles	12.1
A. sundaicus Rodenwaldt	5.8
A. varuna Iyengar	2.5
A. aconitus Dönitz	0.9
A. pallidus Theobald	0.2
A. ramsayi Covell	0.1
A. philippinensis Ludlow	0.03
A. culicifacies Giles	0.01
A. tessellatus Theobald	0.003

TABLE 4

The number of human habitations examined, the total anopheline catch, and the percentage represented by each species, presented on a monthly basis to indicate seasonal prevalences

Collections at Ludlow, Gagalbhai, and 10 villages from August, 1951 through July, 1952 are combined

MONTHS 1951–52	NO. HOUSES EXAM.	TOTAL HOUSE CATCH	*barb.*	*hyr.*	*vag.*	*sub.*	*varu.*	*sund.*	*ann.*	*acon.*	*phil.*	*ram.*	*pall.*	*culic.*	*less.*
Aug.	93	485	10.7	20.8	40.8	10.9	3.9	9.9	2.7	0.2	0.0	0.0	0.0	0.0	0.0
Sept.	115	768	9.2	17.3	41.3	10.0	3.6	14.3	3.8	0.3	0.1	0.0	0.0	0.0	0.0
Oct.	180	934	5.4	5.8	18.5	5.0	6.3	40.0	17.2	1.5	0.0	0.2	0.0	0.0	0.0
Nov.	216	834	10.7	10.3	19.3	2.9	5.0	31.9	15.3	4.1	0.0	0.0	0.5	0.0	0.0
Dec.	192	631	11.7	10.8	32.3	3.6	7.1	18.5	10.6	3.8	0.3	0.0	1.0	0.2	0.0
Jan.	163	336	14.9	3.6	48.2	3.9	7.1	9.8	10.7	1.5	0.0	0.3	0.0	0.0	0.0
Feb.	171	386	16.8	2.6	44.8	9.8	4.1	9.8	11.7	0.3	0.0	0.0	0.0	0.0	0.0
Mar.	187	485	8.5	1.4	32.4	41.4	0.2	5.6	10.3	0.2	0.0	0.0	0.0	0.0	0.0
Apr.	191	487	2.1	0.8	35.7	57.5	0.8	1.8	0.8	0.4	0.0	0.0	0.0	0.0	0.0
May	181	645	0.8	0.8	35.2	60.8	0.0	1.9	0.4	0.1	0.0	0.0	0.0	0.0	0.0
Jun.	164	657	0.0	1.4	18.3	75.8	0.0	3.8	0.6	0.0	0.0	0.2	0.0	0.0	0.0
Jul.	179	791	0.5	3.5	33.5	52.2	0.3	9.2	0.5	0.0	0.0	0.0	0.0	0.1	0.1
Totals	2032	7439	6.9	6.9	31.3	27.7	3.2	15.2	7.3	1.1	0.04	0.05	0.1	0.03	0.01

TABLE 5

The number of cattlesheds examined, the total anopheline catch, and the percentage represented by each species of mosquito, presented on a monthly basis to indicate seasonal prevalences

Collections at Ludlow, Gagalbhai and 10 villages from August, 1951 through July, 1952 are combined

MONTHS 1951-52	NO. SHEDS EXAM.	TOTAL SHED CATCH	*barb.*	*hyr.*	*vag.*	*sub.*	*varu.*	*sund.*	*ann.*	*acon.*	*phil.*	*ram.*	*pall.*	*culic.*	*tess.*
Aug.	75	969	26.7	33.0	16.5	6.7	2.9	2.4	11.8	0.0	0.0	0.0	0.0	0.0	0.0
Sept.	95	1583	30.6	30.8	11.8	6.9	3.8	2.3	13.4	0.2	0.0	0.1	0.0	0.0	0.0
Oct.	177	3047	20.7	24.7	3.2	1.8	2.5	6.4	39.4	1.2	0.0	0.03	0.06	0.0	0.0
Nov.	211	3518	24.2	20.4	3.5	1.8	4.3	7.1	35.1	2.5	0.3	0.2	0.6	0.0	0.0
Dec.	176	3174	26.1	17.7	9.2	3.3	6.1	5.7	28.2	2.7	0.03	0.3	0.6	0.0	0.0
Jan.	173	2367	38.8	11.2	12.5	7.6	5.0	2.2	21.5	0.7	0.0	0.2	0.4	0.0	0.0
Feb.	184	2151	46.6	6.8	6.6	11.6	1.9	2.7	23.3	0.4	0.0	0.09	0.09	0.0	0.0
Mar.	187	2729	38.9	3.9	6.6	25.0	1.2	1.6	21.8	0.8	0.0	0.04	0.0	0.04	0.0
Apr.	192	2630	12.4	3.5	19.6	57.2	0.2	0.6	5.5	1.1	0.0	0.0	0.0	0.04	0.0
May	208	3277	1.8	8.0	13.6	75.3	0.06	0.5	0.5	0.3	0.0	0.0	0.0	0.0	0.0
Jun.	188	2872	0.7	7.2	7.3	83.4	0.0	0.9	0.5	0.0	0.0	0.03	0.0	0.0	0.0
Jul.	198	3006	2.7	8.7	9.9	70.5	0.07	6.8	0.9	0.0	0.0	0.4	0.0	0.03	0.0
Totals	2064	31323	20.8	13.4	9.4	31.9	2.3	3.5	17.4	0.9	0.03	0.1	0.2	0.01	0.0

No previously unreported species of anophelines were encountered during this study and the list above corresponds exactly (except for the frequency data) to that reported by Sen (1937) from his smaller study of the area between Calcutta and Uluberia. The culicine fauna taken incidentally during the routine collections included 4 genera and 8 species, listed here in order of prevalence: *Culex fatigans*, *C. tritaeniorhynchus*, *C. gelidus*, *C. vishnui*, *Armigeres obturans*, *Mansonioides annulifera*, *M. uniformis*, and *Aedes aegypti*.

The relative prevalence of the several anopheline species taken in human habitations and their seasonal distribution is shown month by month in Table 4. *A. vagus* and *A. subpictus* predominated and accounted for 31.3 and 27.7 per cent respectively of the house catch. *A. sundaicus*, which ranks third with a total of 15 per cent of the house catch, is, however, of greatest significance as regards seasonal distribution. During the autumnal rise in malaria rates among the workers (see below), up to 40 per cent of all mosquitoes taken from houses were of this one species. No other species represented in Table 4 follows a seasonal pattern which can be correlated with the seasonal changes in the incidence of malaria among the workers. Table 5 summarizes the relative prevalence and seasonal changes in prevalence of the anopheline species taken while resting in cattlesheds.

Density of anophelines in DDT-controlled and reference areas.

Since living quarters on the Ludlow premises have been subjected to DDT-spraying since 1948 it is of interest to compare the results of mosquito catches from this area with those from the unprotected Gagalbhai lines and from the unsprayed villages nearby. Dwellings within the Ludlow compound have been sprayed three times yearly (April, July and November) with DDT (50 per cent Geigy wettable powder in water with a final concentration of 2.5 per cent DDT) at a rate of application calculated to yield a deposit of 170 mg. of DDT per square foot of surface. The DDT has been applied by means of an electrically powered pump under the supervision of an experienced member of the public health crew; the execution of this program was not interfered with or interrupted during the course of the study reported herein.

Table 6 offers in summary form a comparison of Ludlow, Gagalbhai, and the villages with respect to mean catch of mosquitoes per 15 minutes per dwelling unit and per cattleshed. For the year, the mean catch at Ludlow averaged 0.2 mosquitoes per 15 minutes per house while the Gagalbhai average was 3.0 and that for the villages was 6.1. There were thus 15 times as many anophelines in the dwellings at Gagalbhai as at Ludlow, and 30 times the Ludlow concentration in the villages. A comparison of the cattleshed catches from the same areas is also of interest, particularly since the sheds at Ludlow had never been subjected to DDT spraying. The data in this regard in Table 6 show that no differences existed between Ludlow and Gagalbhai shed collections and only a 3-fold difference between either of these and the villages. The results embodied in these two sets of data are excellent evidence of the efficacy of DDT in radically reducing anopheline densities in human habitations located in the midst of an uncontrolled environment supporting a large anopheline population.

TABLE 6

Mean catch of all anopheline species per 15 minutes per structure. A summary-comparison of mosquito densities in Ludlow structures with dwellings and sheds at Gagalbhai and in the villages

TYPE OF STRUCTURE	LUDLOW LINES			GAGALBHAI LINES			VILLAGES		
	No. examined	No. mosq. taken	Mean catch	No. examined	No. mosq. taken	Mean catch	No. examined	No. mosq. taken	Mean catch
Houses	620*	118	0.2	405	1219	3.0	1007	6102	6.1
Cattlesheds	223	1287	5.8	58	345	5.9	1783	29691	16.7

* DDT-sprayed. All other structures in table were not DDT-treated. See text for details.

Distribution of A. sundaicus *in the area.*

Anopheles sundaicus is not uniformly distributed in the areas surrounding Ludlow although the mosquito was taken at one or another time from nearly all the collecting locales. The heaviest densities of *A. sundaicus* in human habitations (expressed as the mean catch per 15 minutes per house for the year) occurred in Chengail South (2.1), Beltolla (2.0), the Gagalbhai lines (0.8), and Jelapara (0.6), which, taken together, form an arc around the Ludlow compound (0.008) on the west and north (see Fig. 1). Analysis of the cattleshed catches for the year revealed a similar distribution pattern; it is noteworthy in this case, however, that the unsprayed cattlesheds at Ludlow contained about the same mean density of *A. sundaicus* (0.4) as did those at Gagalbhai (0.5), although both were considerably less infested than were some of the villages. The present distribution of *A. sundaicus* is thus somewhat different from that reported by Iyengar (*loc. cit*) who found the heaviest concentrations in and near Rajbansapara to the east of Ludlow.

Dissections of anophelines for natural infection.

During the course of the year's operations 528 *A. sundaicus* taken from human habitations were dissected and 4 (0.76 per cent) were found infected. The details of these dissections are set forth in Table 7. All but one of the infected specimens were found during the peak malaria months and all of the infected mosquitoes came from those collecting areas where the *A. sundaicus* concentrations had been heaviest. One infected mosquito was found in a small group available for dissection in February and serves to illustrate that this mosquito is probably responsible for the out-of-season transmission as well as for the heightened incidence of malaria during October, November and December. Dissections of a limited number of 6 other species of anophelines were conducted during the year as follows: *A. annularis*, 160; *varuna*, 34; *pallidus*, 7; *philippinensis*, 3; and *ramsayi*, 2. All these dissections proved negative.

MALARIA IN THE LABOR FORCE

The daily pressure of dispensary attendance is usually such that each worker can be allotted but little time and attention, and, until the beginning of this

TABLE 7

The results of dissections of Anopheles sundaicus *taken from human habitations during routine catches from August, 1951 through July, 1952*

MONTHS 1951-52	LUDLOW	GAGALBHAI	BELTOLLA	JELAPARA	CHENGAIL SOUTH	CHAK-KASI	RAJBAN-SAPARA	SISBERIA	TOTAL	GUT POSITIVE		GLAND POSITIVE	
										No.	%	No.	%
Aug.	—	2	17	3	—	—	—	—	22	0	0.0	0	0.0
Sept.	—	7	23	5	13	1	1	—	50	0	0.0	0	0.0
Oct.	—	30	65*	—	35	—	—	2	132	1	0.7	0	0.0
Nov.	—	38*	32	5	33	1	—	—	109	0	0.0	1	0.9
Dec.	2	22	23*	6	14	—	—	—	67	1	1.5	0	0.0
Jan.	—	11	2	13	—	—	—	—	26	0	0.0	0	0.0
Feb.	—	13	—	10*	1	—	—	—	24	1†	4.1	1†	4.1
Mar.	—	11	—	1	5	—	2	—	19	0	0.0	0	0.0
Apr.	—	6	1	—	1	—	—	—	8	0	0.0	0	0.0
May	—	8	1	—	—	—	—	—	9	0	0.0	0	0.0
Jun.	—	7	3	—	2	1	1	—	14	0	0.0	0	0.0
Jul.	—	28	11	2	5	—	2	—	48	0	0.0	0	0.0
	2	183	178	45	109	3	6	2	528	3	.57	2	.38

* Indicates source of infected mosquito

† Same mosquito

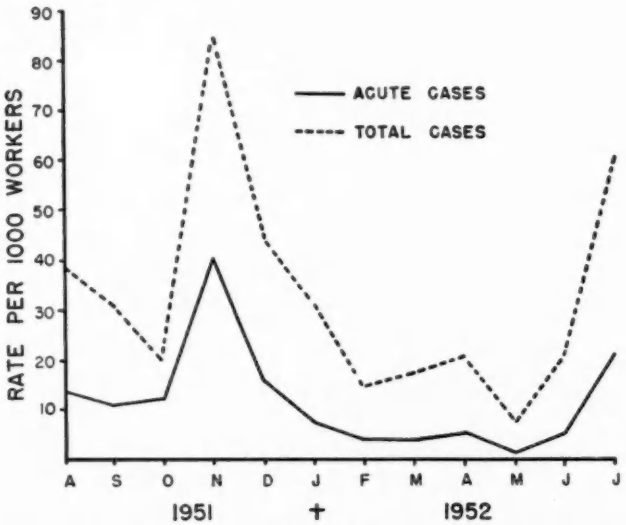

FIG. 3. Malaria rates among Ludlow workers during the year August, 1951–July, 1952. Rates are based on medical leaves issued.

project, few if any blood smears were taken for microscopic examination from patients suspected of having malaria. Treatment was instituted with a variety of the newer antimalarials, and medical leaves were issued liberally when malaria was diagnosed. The objective circumstances are such, however, that with the exception of the clinically overt case, a considerable margin of diagnostic error must be allowed for in which the clinical abilities and experience of the individual physician play a large role.

Records of malaria rates and blood smears examined.

During the year under consideration a total of 2,655 cases were diagnosed as malaria and given leave, of which 960 were listed as acute. The peak was reached in November when a case rate of 84.4 per 1,000 workers was recorded, nearly half of which were considered to be acute (Fig. 3). This rate was double that recorded for the same period during the preceding year and reflected the greatest rise in malaria incidence since 1945. From the beginning of the current studies at Ludlow, the medical staff were encouraged to submit for microscopic examination as many blood smears as their work would permit them to prepare. All three physicians cooperated in this effort to about the same degree and no attempt was made to interfere in any way with their selection of cases. The results of the 988 blood smear examinations (thick and thin film from each case) are presented in Fig. 4 and it is evident from this that the trend in the percentage of microscopically positive smears follows a seasonal pattern paralleling that of the malaria rates in general. During the malaria season as many as half of the slides submitted proved positive and, considering that only a single film was examined

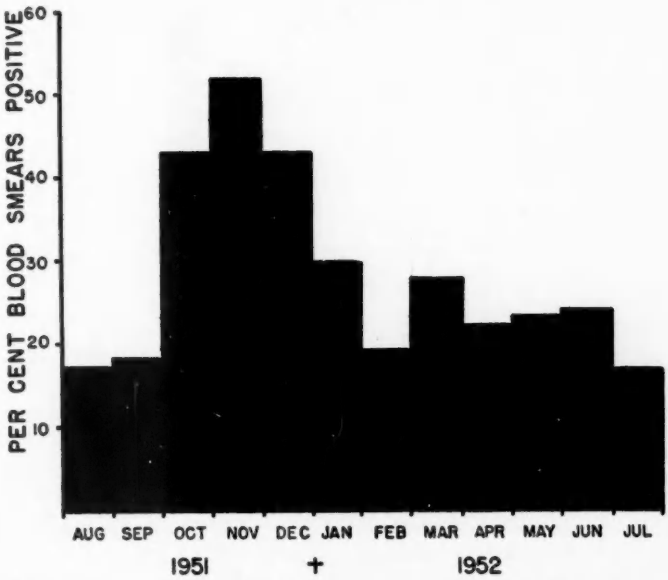

FIG. 4. The percentage of positive blood smears taken from Ludlow workers suspected of having malaria. Based on 988 microscopic examinations.

from each case, this represents a very high level of clinical accuracy. However, before and after the peak months the percentage of positive slides becomes much reduced, indicating the markedly higher probabilities of misdiagnosis during the off-season months. In all, however, a yearly average of 36 per cent positive findings on routine, single-smear examinations represents a greater degree of accuracy in clinical diagnosis than might have been anticipated under the circumstances. *P. vivax* was found in 66 per cent of the positive slides and *P. falciparum* made up most of the balance, except for 1.3 per cent represented by *P. malariae*. No mixed infections were encountered during these examinations.

Malaria rates and place of residence.

Considering the geographic distribution of the workers and the fact that only the compound quarters are sprayed with DDT, it is of value to compare the malaria rates among those resident in the compound with the group living in the villages. Fig. 5, based on pharmacy records[1], illustrates the marked difference in the two groups, the rates being a third higher in the village habitants. Similarly it is of considerable interest to compare the rates at Ludlow (where one-half of the labor force lived in DDT-sprayed quarters) with the worker population of

[1] For several reasons, pharmacy records show higher rates than do those based on leaves issued. However, only the pharmacy records indicate place of residence and the interest here lies in the relative proportions of compound to village rates rather than in the absolute values of the rates themselves.

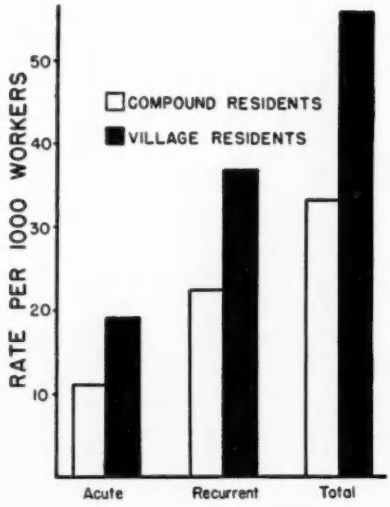

FIG. 5. Total malaria rates (1951–1952) among compound-resident Ludlow employees as compared with those resident in the villages.

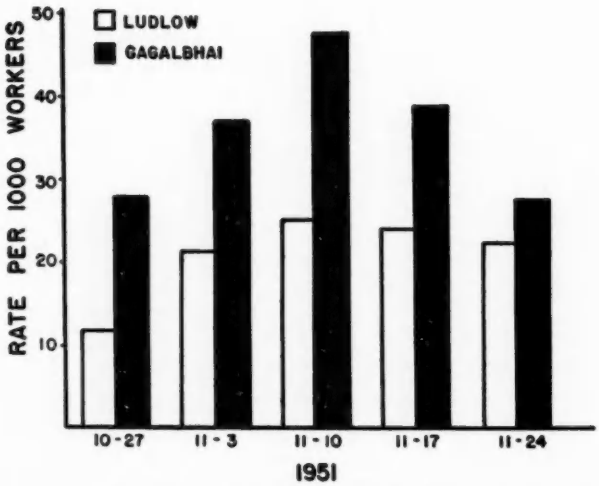

FIG. 6. A comparison of total malaria rates among Ludlow workers with rates among Gagalbhai employees during the peak weeks of the malaria season.

the adjacent Gagalbhai mill. Gagalbhai employs some 2,300 workers, less than half of whom live in lines located close to the western border of the Ludlow compound. During the peak weeks of the malaria season in 1951 the rates among the Gagalbhai workers were very nearly double the rates of the Ludlow workers (Fig. 6), and, as has already been noted, a focal epidemic of malaria was encountered during the second spleen and blood survey. Finally, it may be mentioned that for the first time in 10 years a case of malaria occurred among the persons of the Ludlow supervisory staff. This individual had not been taking paludrine and the circumstances all suggested the probability that the infection (which was confirmed microscopically) was acquired during the course of a night-time visit to another mill where neither DDT nor screens were in use.

Loss of time sustained through malaria.

During the year under consideration, 52,001 attendances were registered for all causes at the dispensary, 17,925 leaves were granted, and 55,721 man-days were lost. Of these totals, 3,408 leaves were issued for malaria (19.0 per cent of all leaves issued) and malaria claimed 7,173 man-days (12.9 per cent of all days lost). These figures are amplified in Table 8 and serve to confirm the seasonal data evidenced by the data presented previously. During the worst of the malaria months (November), 33.5 per cent of all leaves issued and nearly 25 per cent of all days lost were due to malaria alone. These are cold figures and do not begin to encompass the less tangible losses such as time lost in visits to the dispensary, the costs of medical attention and drugs, the toll of energy and productive capacity lost during and after sieges of malaria, and the effects on the general well-being and morale of the workers themselves.

TABLE 8

Medical leaves granted and man-days lost due to malaria as compared with leaves issued and man-days lost for all medical reasons at Ludlow, August, 1951–July, 1952

MONTHS 1951–52	DISPENSARY CASES—ALL TYPES			MALARIA ALONE			
	Total attend.	Total given leave	Total days lost	Total given leave*	% of all leaves	Total days lost	% of all lost
Aug.	5773	1590	5068	326	20.5	691	13.6
Sept.	6724	2433	6931	268	11.0	532	7.7
Oct.	3806	1038	3099	200	19.3	656	21.2
Nov.	5752	2356	7286	789	33.5	1793	24.6
Dec.	5099	1931	5334	401	20.8	721	13.5
Jan.	3238	1133	3503	281	25.8	567	16.2
Feb.	3226	885	3012	106	11.9	211	7.0
Mar.	3695	1176	3919	122	10.4	113	2.9
Apr.	3270	1246	4283	177	14.2	398	9.3
May	2554	709	2514	77	10.9	160	6.4
Jun.	3882	1473	4728	182	12.4	182	8.1
Jul.	5982	1955	6044	479	24.5	950	15.7
Totals	52001	17925	55721	3408	19.0	7173	12.9

* Including those receiving more than one period of leave.

Prophylaxis against malaria has been freely available to the workers at Ludlow since 1949. A medical department team visits each section of the mill once weekly offering paludrine tablets to all who agree to take them on the spot, but the results have been disappointing. Records of paludrine distribution indicate that less than 10 per cent of the workers accept prophylaxis and that many of these do not avail themselves of the drug each week.

DISCUSSION

As a result of the DDT project currently operative at Ludlow, the compound may be regarded as a comparatively sheltered area in the midst of an uncontrolled environment. Despite the control in the mill area, however, it is obvious from the data that a) malaria still represents a problem to the company and the community, and that b) even persons domiciled within the protected locale fall victim to malaria, although with less frequency than their village-resident counterparts. There are several elements which are important to the epidemiology of malaria as it occurs within the compound and which serve to explain such cases as do occur there.

Although no anophelines were ever found breeding within the compound, the 8-foot wall surrounding it may hardly be considered a barrier to the ingress of mosquitoes from nearby villages and breeding waters. The data referring to catches from the unsprayed cattlesheds at Ludlow (Table 6) attest to the fact that mosquitoes do indeed enter the compound and to the same degree as they enter the totally unprotected Gagalbhai environment. That they are only rarely found inside Ludlow's human habitations is, of course, significant, but the significance of this observation must be tempered with at least three other considerations. The first is that most of the line-resident workers sleep outdoors as long as the weather remains reasonably warm. During the period of the study it was observed that many were still sleeping in the open as late as October 25, just 2–3 weeks prior to the peak incidence of malaria among them. Even in November beds were occasionally moved from the rooms to the streets where invading *A. sundaicus* could readily find blood meals, although the mosquitoes might subsequently be killed by resting indoors on DDT-treated surfaces. The second consideration is that the mill-resident workers are in no way restricted to the confines of the compound and are completely at liberty, outside of working hours, to visit the lines of other mills, the surrounding villages, or the nearby bazaars—and in the process be exposed to increased risks of acquiring malaria. The third factor of epidemiological significance is that the mill resident workers, being of exogenous origins, may make the long trip one or more times during the course of the year to their provincial homes, where, of course, they are subject to the malaria risks peculiar to their home communities. Since leaves are usually for a 2-week period, they return to the mill at, or shortly before, the time that incipient, newly-acquired cases of malaria would become overt; thus, the local burden of malaria cases is increased and new strains of malaria are constantly being imported from other parts of India.

The accumulated evidence of this and earlier studies seems to leave little

doubt that *A. sundaicus* is the primary vector in the Chengail area, but the mosquito has obviously not succeeded in maintaining itself at or near the level that produced the 1930 epidemic reported by Iyengar (*loc. cit.*). The ecological bases for this decline are not known, but far from exceeding "in number all other local species of anophelines many times over" (as Iyengar described it), *A. sundaicus* now constitutes, at the very peak of its annual cycle, some 40 per cent of the anophelines taken from houses (Table 4). Even at this level, considering the reported efficiency of the species, one can only speculate as to why the local degree of endemicity is so low as to be barely measurable—unless it be that the absolute numbers of vector mosquitoes is too small for transmission on a greater scale. With all this, it should be borne in mind that as long as *A. sundaicus* remains established in the inland brackish waters, the potential threat of a sudden increase in the incidence of malaria remains everpresent.

The current study of the malaria problem at Ludlow revealed several salient points which may be reconsidered briefly. Generally, the Chengail area was found to be one of very low malaria endemicity, although a fairly serious outbreak was uncovered at Gagalbhai. The malaria rates for mill-resident Ludlow workers were lower than those of their village-resident counterparts (Fig. 5), and the Ludlow workers as a group sustained a lower rate than those employed at Gagalbhai (Fig. 6). These differences are primarily attributed to the DDT control measures within the Ludlow compound dwellings as a result of which few anophelines could be taken from Ludlow houses as compared with catches from Gagalbhai habitations (Table 6). These differences in anopheline densities as a whole and in *A. sundaicus* densities in particular are considered to be a direct product of the control measure, and not of such extraneous factors as the relative distances from breeding areas; this is borne out by the fact that there was no difference in the catches from Ludlow cattlesheds as compared with catches in the same category from Gagalbhai, either with regard to all anophelines or with specific reference to *A. sundaicus*.

The observations embodied in this report indicate the relative efficacy of the control measures employed to date at Ludlow, but they also serve to illustrate the limitations of an effort designed to exercise control over a restricted area in the midst of totally uncontrolled surroundings, even in a place where the incidence of malaria is comparatively low. It is obvious that the goal of malaria reduction beyond the present level will not be attained until the whole of the environment comes under some semblance of control, and until the worker who lives eight miles distant enjoys the same measure of protection as the one who lives within the compound walls. Furthermore, it is only when that distant worker's environment becomes adequately controlled that those resident within the compound will be assured of maximal protection.

SUMMARY

Under the auspices of the Ludlow Jute Co., Ltd., an investigation into the epidemiology and local significance of malaria was conducted at the company's jute mill in India from August, 1951, through July, 1952. This mill is located on

the banks of the tidal Hooghly River, 17 miles south of Calcutta, and employs 6,000–7,000 workers half of whom (together with dependents) are quartered within the mill compound, the remainder residing in nearby villages. Dwellings within the Ludlow compound have been sprayed with DDT thrice yearly since 1948; an immediately adjacent mill (Gagalbhai) and the nearly villages served as nonprotected reference areas for the study.

Two spleen and blood-smear surveys among children at the mills and in the villages revealed a low incidence of malaria, although a focal outbreak was uncovered at Gagalbhai where the spleen index rose to nearly 15 per cent and the parasite index to 58 per cent; at Ludlow and in the villages, however, both indices remained well below 5 per cent.

Regular mosquito catches (in the two mill areas and in 10 villages) disclosed 13 species of anophelines. *Anopheles sundaicus* predominated in catches from human dwellings during the height of the malaria season (October–November), accounting for as much as 40 per cent of the total house catch. Naturally infected *A. sundaicus* were taken during October–December and again in February and a natural infection rate of 0.76 per cent was established for the year; no other species of anopheline was found naturally infected. Mean-unit mosquito catches from Ludlow dwellings were 15 times lower than those from Gagalbhai and 30 times lower than those from the villages. Only rare specimens of *A. sundaicus* were taken in Ludlow compound dwellings while outside the compound the mosquito was found in focal concentrations.

The malaria season of 1951–1952 was a comparatively severe one locally. At Ludlow during November the malaria rate based on medical leaves issued reached 84 per 1,000; however, at Gagalbhai the rate was very nearly double that for the same period. Malaria rates were also lower among Ludlow compound residents as compared with village-resident Ludlow employees.

The economic significance of malaria at Ludlow may be judged from the following data: during the year 3,408 leaves were issued for malaria (19.0 per cent of all medical leaves) and these resulted in a loss of 7,173 man-days (12.9 per cent of all time lost to illness). In November, the peak malaria month, 33.5 per cent of the medical leaves issued and 24.6 per cent of the days lost were attributable to malaria.

The epidemiology of local malaria is described and the merits and shortcomings of current control operations are discussed.

Acknowledgements

This project was supported by the Ludlow Manufacturing and Sales Company of Boston in cooperation with the Harvard School of Public Health. I should like to express my appreciation to Mr. Peter L. Stone, vice-president of the company, to the managerial and supervisory personnel of the Chengail mill, and to the executive staff of the Calcutta office of the Ludlow Jute Co., Ltd., for their sincere interest in the studies. I am indebted to Dr. K. L. Basu Mallick, lately Chief Medical Officer at the Chengail mill, and to the members of his staff, for their generous cooperation during the course of this project, and to Mr. D. H. Robertson for permission to carry out phases of the study at the Gagalbhai mill. I should also like to express my thanks to Dr. D. L. Augustine, Harvard School of Public

Health, and Dr. K. V. Krishnan, All-India Institute of Hygiene and Public Health, for their encouragement and advice, as well as to the many staff members of the latter institution and of the Calcutta School of Tropical Medicine whom I had occasion to consult. Finally, grateful acknowledgement is made of the services of Mr. Ravi Iyengar, entomological assistant, and Mr. N. N. Chatterjee, laboratory technician, for their capable and untiring assistance throughout the year.

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PROBLEMS IN TROPICAL PUBLIC HEALTH AMONG WORKERS AT A JUTE MILL NEAR CALCUTTA

II. A Study of Intestinal Parasites in the Labor Force

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This report presents the results of a survey on infections with intestinal parasites conducted among 750 workers at the mills of the Ludlow Jute Co., Ltd., Chengail (Howrah District, West Bengal), during the premonsoon period from March–June, 1952. Observations made concurrently with this study will form the basis for subsequent reports dealing with hookworm infection and its relationship to hemoglobin levels, differential white cell counts and the problem of eosinophilia, and with the results of parasitologic and hematologic studies conducted among the members of the mill's supervisory staff, their families, and their foodhandler servants.

A previous publication (Chernin, 1954), concerned with the problem of malaria in the mill population, may be referred to for a detailed description of the local environment and topography as well as for weather data encompassing the period during which the present study was in progress.

MATERIALS AND METHODS

A group of 10–15 workers was sent to the laboratory from the mill each morning at 7:30 A.M., each having been instructed by his department supervisor on the previous day not to pass the morning stool until arrival at the dispensary. After blood smears were prepared and hemoglobin determinations recorded, the stools were passed in clean enameled containers in a latrine immediately adjacent to the laboratory. Workers who could not pass stools at this time were detained for an hour or more and requested to try again; those who failed at the second attempt were instructed to report to the dispensary on the following morning.

All records were maintained on specially designed punch-cards (Fig. 1). The central portion of the card (pertaining to age, sex, religion, etc.) was prepared by the department supervisor in consultation with the worker on the day prior to his scheduled visit to the laboratory. The information was then checked as each worker presented his card at the laboratory and the data subsequently punched into the appropriate marginal positions. The findings derived from the stool and blood examinations were entered on the card by pencilled entries or marginal notations and later punched out for ease in sorting and analysis.

Stool specimens were examined within 20 minutes of the time they were passed in order to obviate the possibilities of organisms degenerating. Macroscopic examination of each specimen was made to observe consistency and to note the presence of blood, mucus, or spontaneously discharged helminths. One fecal

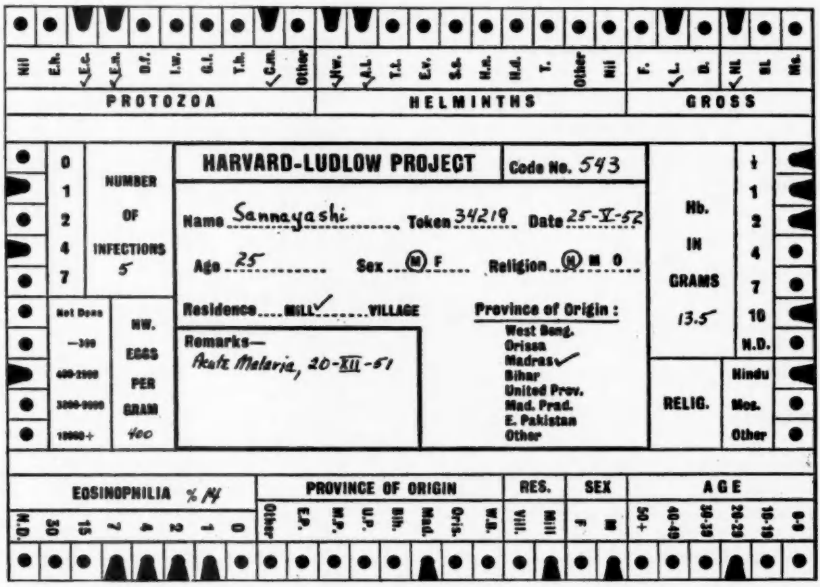

FIG. 1. Sample punch-card used for recording and analyzing the results of the intestinal parasite and hematologic survey.

smear in saline and one in Velat's stain (Velat *et al.*, 1950) were prepared from each specimen and examined microscopically. Each stool was further examined by means of a zinc sulfate concentration specimen carefully prepared by centrifugation, collected by the coverslip technic and mounted for examination in dilute iodine (Lugol's solution); hookworm egg-counts were done by the Stoll method whenever that infection was encountered. In all cases only a single stool specimen from each individual was examined.

COMPOSITION OF THE SAMPLE GROUP

Between 6,000 and 7,000 workers are regularly employed at the Ludlow Jute Company mill. Approximately half of the workers are of indigenous (Bengali) origin and live in nearby villages where sanitation is of a very primitive order; the remainder, composed almost entirely of persons from provinces other than West Bengal, live within the company compound in workers' quarters ("lines") which are provided with sanitary facilities to be described in detail later. The survey sample includes, so far as it proved practicable, the same proportions of the sexes, the two religious groups (Hindus and Muslims), and provincial groups as were indicated by company census figures. Since inquiries revealed that the various elements of the population were evenly distributed throughout the mill, the supervisors in one portion of the mill were asked to select workers at random for purposes of the survey. In order to randomize still further, the supervisors

TABLE 1

General composition of the population sample of 750 workers surveyed for intestinal parasite infections at the Ludlow mill

GROUPS REPRESENTED	IN SURVEY SAMPLE		COMPANY CENSUS ESTIMATE (PER CENT)
	Number	Per cent	
Males	645 (750)	86.0	92.6
Females	105	14.0	7.4
Mill Residents	384 (750)	51.2	51.0
Village Residents	366	48.8	49.0
Hindus	566 (750)	75.5	72.3
Muslims	184	24.5	27.7
West Bengal	357	47.6	51.9
Orissa	139	18.5	19.4
Madras	180	24.0	15.5
Bihar	44	5.9	6.7
Madhya Pradesh	15	2.0	1.8
Utter Pradesh	15	2.0	3.5
Totals	750	100.0	98.8*

* The remaining 1.2 per cent is composed of other population groups who are not represented in the survey sample because of their small numbers.

were restricted for the first half of the survey to workers whose company registration numbers ("token" numbers) ended with an odd digit; for the second half of the survey the procedure was reversed and only workers with tokens ending with even digits were selected.

The final composition of the sample group of 750 is presented in Table 1 together with the corresponding percentile figures drawn from the company's current census estimate. In only one case, that of the proportion of females to males, does the sample composition differ markedly from that of the whole population. In this case it was felt that a sample proportion of females corresponding to their 7.4 per cent status in the general census would yield too small a group (about 50 individuals) to permit of meaningful analysis; accordingly, the proportion of females included in the sample was arbitrarily doubled. Since, however, almost all of the females employed by the company come from the province of Madras, the increase in the number of females in the sample resulted in an increase of the relative proportion of Madrassis as a group, a disproportion which is not considered to affect significantly the overall composition of the sample.

The age composition of the sample group is presented in Figure 2. No com-

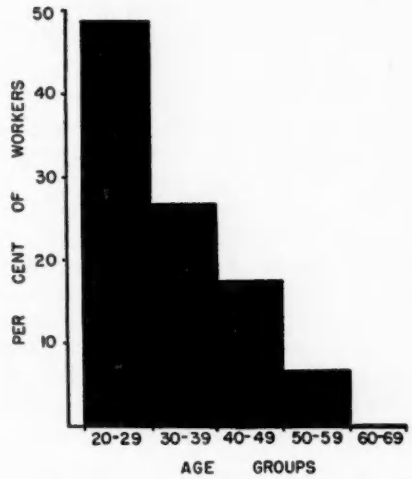

FIG. 2. The age composition of the sample population of 750 persons surveyed for intestinal parasite infections at the Ludlow Jute Company's mill, Chengail, India.,

parable data based on company records are available for the working population as a whole.

CLINICAL RECORDS

The data in Table 2 represent a summary of clinical cases of dysenteries and other gastrointestinal complaints as recorded in the company dispensary during the year from August, 1951, through July, 1952. With rare exception, diagnoses were made without recourse to supportive laboratory studies. It is interesting nevertheless to note the relatively low proportion of dysenteries with respect to other gastrointestinal complaints as well as the relatively minor differences in rates between compound residents as compared with village residents.

TABLE 2

Clinical cases of dysentery and other G.I. complaints as recorded at the Ludlow dispensary for the year from August, 1951 through July, 1952

PLACE OF RESIDENCE	DYSENTERIES		ALL OTHER G.I. COMPLAINTS	
	Number of cases	Rate per 1000	Number of cases	Rate per 1000
Compound	235	6.1	2410	62.6
Village	429	10.7	3526	88.0
Totals	664	8.5	5936	75.6

SURVEY RESULTS

The results of the survey of intestinal parasites among the sample of 750 workers at the Ludlow mill are summarized in Table 3. The commonest of the infections ordinarily considered to be pathogenic proved to be hookworm (68.7 per cent), followed in order of prevalence by: *Trichuris trichiura* (16.4 per cent), *Ascaris lumbricoides* (9.6 per cent), *Giardia lamblia* (9.1 per cent), *Entamoeba histolytica* (8.3 per cent), and *Strongyloides stercoralis* (8.1 per cent). Of the 750 persons, 92.5 per cent were found to be parasitized by one or more organisms, 73.5 per cent with protozoan infections, and 76.1 per cent with one of more helminthic infections; the mean number of infections per person was 2.5.

In general, females were more prone to higher infection rates with protozoa than were males (Table 4), and conversely the males suffered higher rates of infection with almost all of the prevalent helminths. The prevalence of protozoan infections was higher among Hindus than Muslims, while among Muslims almost all of the helminthic infections were of a higher order of prevalence (Table 4). Muslims, as a group, carried an average of 2.9 infections per person while the

TABLE 3

General summary of the prevalence of intestinal protozoan and helminthic infection among 750 workers at the Ludlow Jute Company's mill

The survey was conducted during the period March–June, 1952

PARASITES	WORKERS INFECTED	
	Number	Per cent
Entamoeba histolytica	62	8.3
Entamoeba coli	436	58.1
Endolimax nana	188	25.1
Dientamoeba fragilis	39	5.2
Iodamoeba williamsi	18	2.4
Giardia lamblia	68	9.1
Trichomonas hominis	90	12.0
Chilomastix mesnili	88	11.7
Enteromonas hominis	62	8.3
Total with protozoan infections	551	73.5
Hookworm sp.*	515	68.7
Ascaris lumbricoides	72	9.6
Trichuris trichiura	123	16.4
Enterobius vermicularis	30	4.0
Strongyloides stercoralis	61	8.1
Hymenolepis nana	15	2.0
Total with helminthic infections	571	76.1
Total prevalence—all parasites	694	92.5
Mean number infections per person	2.5	

* Species not determined.

TABLE 4

A comparison of males with females and Hindus with Muslims in regard to the prevalence of intestinal parasite infections

Numbers in brackets under each column heading indicate the number of persons in the group

PARASITES	MALES (645)		FEMALES (105)		HINDUS (566)		MUSLIMS (184)	
	Infected		Infected		Infected		Infected	
	No.	%	No.	%	No.	%	No.	%
Entamoeba histolytica	51	7.9	11	10.5	48	8.5	14	7.6
Entamoeba coli	367	56.9	69	65.7	337	59.5	99	53.8
Endolimax nana	154	23.9	34	32.4	150	26.5	38	20.7
Dientamoeba fragilis	30	4.7	9	8.6	32	5.6	7	3.8
Iodamoeba williamsi	14	2.2	4	3.8	14	2.5	4	2.2
Giardia lamblia	64	9.9	4	3.8	56	9.9	12	6.5
Trichomonas hominis	75	11.6	15	14.3	66	11.7	24	13.0
Chilomastix mesnili	73	11.3	15	14.3	68	12.0	20	10.9
Enteromonas hominis	50	7.8	12	11.4	53	9.4	9	4.9
Total with protozoan infections	468	72.6	83	79.0	423	74.7	128	69.6
Hookworm sp.*	454	70.4	61	58.1	377	66.7	138	75.0
Ascaris lumbricoides	69	10.7	3	2.9	17	3.0	55	29.9
Trichuris trichiura	114	17.7	9	8.6	37	6.5	86	46.7
Enterobius vermicularis	27	4.2	3	2.9	19	3.4	11	5.9
Strongyloides stercoralis	49	7.6	12	11.4	50	8.8	11	5.9
Hymenolepis nana	15	2.3	0	0.0	12	2.1	3	1.6
Total with helminthic infections	503	77.9	68	64.8	405	71.6	166	90.2
Total prevalence—all parasites	601	93.2	93	88.6	515	91.0	179	97.3
Mean number infections per person	2.5		2.5		2.4		2.9	

* Species not determined.

mean number of infections per person for Hindus was 2.4. More interesting, however, than these slight general variances are the striking differences between Hindus and Muslims with specific regard to ascariasis and trichuriasis: among Hindus the respective infection rates were 3.0 per cent and 6.5 per cent, while among Muslims the rates were 29.9 per cent and 46.7 per cent.

The data presented in Table 5 compare the prevalence of the several intestinal parasites among compound residents with village-resident workers, and indicate that few (if any) differences exist between the two groups. While it may appear from the totals that ascariasis and trichuriasis are much more prevalent in the

TABLE 5

A comparison of mill-resident with village-resident employees of the Ludlow Jute Company with respect to the prevalence of intestinal parasite infections

Numbers in brackets under each column heading indicate the number of persons in the group

PARASITES	ALL MILL RESIDENTS						ALL VILLAGE RESIDENTS					
	Hindu (366)		Muslim (18)		Total (384)		Hindu (200)		Muslim (166)		Total (366)	
	Infected						Infected					
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Entamoeba histolytica	30	8.2	2	—	32	8.3	18	9.0	12	7.2	30	8.2
Entamoeba coli	221	60.3	12	—	233	60.7	116	58.0	87	52.4	203	55.5
Endolimax nana	98	26.8	3	—	101	26.3	52	26.0	35	21.1	87	23.8
Dientamoeba fragilis	26	7.1	0	—	26	6.8	6	3.0	7	4.2	13	3.6
Iodamoeba williamsi	8	2.2	0	—	8	2.1	6	3.0	4	2.4	10	2.7
Giardia lamblia	24	6.6	0	—	24	6.3	32	16.0	12	7.2	44	12.0
Trichomonas hominis	36	9.8	3	—	39	10.2	30	15.0	21	12.7	51	13.9
Chilomastix mesnili	38	10.4	1	—	39	10.2	30	15.0	19	11.4	49	13.4
Enteromonas hominis	27	7.4	1	—	28	7.3	26	13.0	8	4.8	34	9.3
Total with protozoan infections	272	74.3	12	66.7	284	74.0	151	75.5	116	69.9	267	73.0
Hookworm sp.*	251	68.6	8	—	259	67.4	126	63.0	130	78.3	256	69.9
Ascaris lumbricoides	14	3.8	2	—	16	4.2	3	1.5	53	31.9	56	15.3
Trichuris trichiura	28	7.7	4	—	32	8.3	9	4.5	82	49.4	91	24.9
Enterobius vermicularis	13	3.6	0	—	13	3.4	6	3.0	11	6.6	17	4.6
Strongyloides stercoralis	38	10.4	0	—	38	9.9	12	6.0	11	6.6	23	6.3
Hymenolepis nana	8	2.2	1	—	9	2.3	4	2.0	2	1.2	6	1.6
Total with helminthic infections	271	74.0	12	66.7	283	73.7	134	67.0	154	92.8	288	78.9
Total prevalence—all parasites	332	90.7	17	94.4	349	90.9	183	91.5	162	97.6	345	94.3
Mean No. infections per person	2.3		2.1		2.3		2.4		2.9		2.7	

* Species not determined.

villages than in the compound, further examination of the tabular data reveals that the differences are not so much a reflection of place of residence *per se* but are rather related primarily to religious affiliation. It has already been noted that the prevalence of the two infections is much greater among Muslims than among Hindus and the population distribution is such that many more Muslims live in the villages than in the compound; furthermore, if one compares Hindus and Muslims living side by side in the villages (Table 5) it becomes evident that ascariasis is 20 times as prevalent and trichuriasis 10 times as prevalent among Muslims as among Hindus.

Some general conception of the burden of parasitic infections in the sample group may be gathered by reference to Figure 3. Only 7.5 per cent of the group was found to be free of all infections, while at the other extreme nearly 10 per cent of those examined proved to have 5 or more (up to 8) infections per person. It is also of interest to consider the total infection rate by age groups as well as the proportions within each age group carrying given numbers of infections. Table 6 summarizes an analysis along these lines and serves to illustrate the small but discrete decreases that occur in the total infection rates with increase in age; even in the oldest age group, however, 85 per cent remain positive for one or more infections. The proportion of persons carrying a given number of infections is subject to surprisingly little variation from one age group to the next.

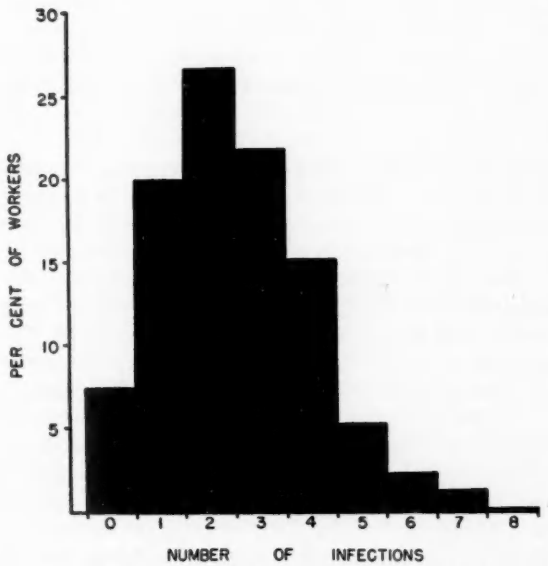

FIG. 3. The percentage of persons with given numbers of intestinal parasite infections. Based on the sample group of 750 persons.

TABLE 6

Total prevalence of intestinal parasite infections by age groups together with the relative proportions within each age group that were found to carry given numbers of infections (based on 750 persons)

Numbers in brackets indicate the number of individuals in each age group

NUMBER OF INFECTIONS	20–29 (367) PER CENT	30–39 (198) PER CENT	40–49 (132) PER CENT	50+ (53) PER CENT
Negative	3.5	9.6	12.1	15.1
1	17.9	24.7	21.2	15.1
2	29.7	22.7	23.5	28.3
3	22.6	21.2	21.9	16.9
4	16.9	14.1	12.1	13.2
5	5.9	3.5	6.1	5.7
6	1.6	2.0	2.3	5.7
7	1.6	1.5	0.8	0.0
8	0.0	0.5	0.0	0.0
Total positive	96.5	90.4	87.9	84.9

DISCUSSION

A search of the literature relevant to intestinal parasite surveys in India from 1925 to date revealed no references which might serve as a basis for direct comparison with the present study. However, Mukerji (1927) reported on 1,524 hospital patients whose stools were examined routinely for helminths on admission to the Carmichael Hospital for Tropical Diseases in Calcutta; hookworm infection was found in 27.6 per cent, ascariasis in 10.7 per cent and trichuriasis in 13.4 per cent of the individuals. These figures are in poor agreement with those of Chatterjee (1927) for a group of patients admitted to the nearby Calcutta Medical College Hospitals in which the respective rates were 11.6 per cent, 5.4 per cent and 3.0 per cent. Neither of the samples can be considered representative of the community and the same is true of a group of 9,015 patients examined for intestinal protozoa upon admission to the hospital associated with the Calcutta School of Tropical Medicine during the decade 1925–1934 (Chaudhuri and Chaudhuri, 1946). In this series, 26.2 per cent were found infected with protozoa, 8.8 per cent with *E. histolytica*, 5.1 per cent with *E. coli*, 6.9 per cent with *E. nana* and 5.2 per cent with *G. lamblia*, with rates for the remaining protozoa falling below 2.3 per cent. With the exception of the *E. histolytica* figure, which corresponds very well with the 8.2 per cent rate herein reported, none of the others is in agreement with the results of the present study. It should be borne in mind, however, that the data cited above were drawn from groups which presumably were predominantly urban rather than rural in origin.

Hookworm infection was found to be widespread in the sample population at Ludlow to the extent of 68.7 per cent. This figure is in general agreement with those Chandler compiled for this portion of Bengal (Chandler, 1926), although Lal and Seal (1949), using a "rough method of examination," reported a somewhat lower figure from a survey of the Singur area just north of Calcutta. De-

spite its wide prevalence, the intensity of infection (as measured by Stoll egg counts) among Ludlow workers is, with rare exception, very low; this finding also corroborates Chandler's conclusions on this point of 25 years ago. The observation just mentioned will be further detailed in a later publication, but for the moment it is of interest to point out that the prevalence of the infection among workers living in the sanitated labor lines (67.4 per cent) very closely approximates that found among workers resident in the surrounding villages (69.9 per cent) where sanitation is poor. The significance of these similarities will become evident later in this discussion.

The prevalence of ascariasis (9.6 per cent) and of trichuriasis (16.4 per cent) in the Ludlow sample are in keeping with figures given by Mukerji (*loc. cit.*) and, in general, with those given by Chandler (*loc. cit.*). However, it has been pointed out that there is a marked difference between Hindus and Muslims in the sample with regard to these infections (*see* Table 4), and further, that in the village environment ascariasis is 20 times as prevalent and trichuriasis is 10 times as prevalent among Muslims as Hindus (*see* totals for Hindu and Muslim village residents, Table 5). No ready explanation for these differences presents itself, particularly since such marked discrepancies between the two religious groups are not evidenced for any other infections. Chandler (1928) has observed that "in Bengal . . . where the rainfall is heavy and surface water abundant during the monsoon season, and where polluted ground tanks are used both for bathing and drinking, both *Ascaris* and *Trichuris* infections are very common." This suggestion goes far in explaining the general prevalence of the two infections in the population at large, but it would not help to clarify the differences noted between Hindus and Muslims unless it were to be established that water sources are not used in common and that those utilized by Muslims were more heavily polluted. If, however, the latter supposition is assumed to be valid, then one could reasonably expect that Muslims would have higher rates than Hindus for most of the protozoan infections as well as for ascariasis and trichuriasis; actually, Hindus and Muslims are infected with protozoa approximately to the same extent (Tables 4 and 5). One general observation which has been made concerning the habits of the two groups is that the sanitation of the immediate environment, and particularly that of the kitchen environment, of Muslim houses was decidedly inferior to that seen among Hindus. The possible importance of this to the epidemiology of ascariasis and trichuriasis in an adult population must await more detailed studies than were possible when the data were originally collected.

Sanitary conditions within the Ludlow mill compound and its labor lines, while perhaps somewhat inadequate by absolute standards, are nevertheless vastly superior to those that obtain in the surrounding villages. Nine permanent latrines (automatic flush) with a total of 126 places and a capacity of 2700 users serve an estimated 4500 persons in the lines; three latrines (159 places) with a lapacity of 7000 users serve the personnel in the mill area during working hours. There are, in addition, 6 urinals in the lines and 4 in the mill area, with a combined total of 97 places. These facilities are supplemented by a system of sewage

pipes and by septic and treatment tanks from which the effluent is eventually pumped into the nearby Hooghly River. The streets and lanes in the lines are brick-paved and provided with surface drains, and a squad of sweepers, under direct supervision of the company's medical staff, is responsible for maintaining the cleanliness of the latrines, thoroughfares and the surrounding environment. The drinking water supply for the lines is drawn from the Hooghly (a tidal river), during and for a short time after the monsoon season when the salinity of the river water is at its lowest. This water is then stored for use during the balance of the year in a series of large, open reservoirs within the compound. It is passed through a Jewell sand-filter system, chlorinated automatically, pumped to an overhead tank and delivered by pipes to sites in the labor lines where a total of 61 taps are provided. There are also 8 tube wells in the lines with a total of 14 taps. Samples of drinking water are sent monthly to the public health laboratories in Calcutta for bacteriological examination and only those sources certified as safe are continued in use. The lines are also provided with 23 public showers (65 taps in all) for the use of the workers and their dependents; water used for this purpose is unfiltered and untreated and is pumped directly from the river. While promiscuous urination was not uncommonly observed in the vicinity of the lines, at no time during the course of a year's observation was there any evidence of gross fecal pollution in the mill or line areas.

The villages are essentially without sanitation. Drinking water is drawn from ditches, tanks, borrow-pits and canals convenient to the houses. These sources normally also serve for bathing and ablutions, laundering, and for the watering of stock, and they are only rarely supplemented by tube wells. Only the few houses of the comparatively well-to-do have their own latrines; otherwise, it is common for the members of the family to share a defecating area in the vicinity of the house. These sites are ordinarily near water and some of them are provided with makeshift foot-rests or "squatting places" of brick or bamboo which serve in some measure to protect the feet against direct contact with fecal matter. Workers in the paddy fields and persons en route from village to village have no recourse but to defecate as circumstances dictate and little, if any, control is exercised over the defecating habits of children. Hygiene on a personal basis is surprisingly good; sanitation on a community or environmental basis is all but nonexistent.

Considering the objective circumstances as just outlined, one might at first suspect that the prevalence of the several intestinal parasites would be considerably higher among village-resident workers than among their line-resident opposites. Table 5, however, clearly illustrates that this is not the case; in fact, the prevalence rates for individual infections and for all taken together are much the same for the two groups of employees. A combination of factors may serve to explain why the two residence groups, separated by such obvious dissimilarities in sanitary facilities, proved to have such strikingly similar parasite rates: the workers who live in the sanitated labor lines undoubtedly acquired many of their infections in their native provinces or elsewhere prior to taking up residence in the Ludlow lines at the time of their employment; furthermore, the

workers commonly make one or more visits home each year and are, of course, exposed to whatever conditions of sanitation prevail while en route and at home; finally, since these workers are free to come and go as they choose during non-working hours, visits to nearby villages and bazaars are commonplace, with the result that they are continually placing themselves outside the pale of developed community sanitation. The same general factors, among others, are also of importance to the epidemiology of malaria as it occurs among workers living within the DDT-sprayed lines (Chernin, 1954).

These considerations all seem to lead inevitably to the conclusion that focal sanitation, no matter how well conceived and executed, cannot of itself be effective in an unconfined and uneducated population in the face of an environment in which sanitation is of a low order or is totally nonexistent. In other words, the "vertical" concentration of sanitation in a small area whose uninformed (sanitary-wise) residents freely circulate in the outside environment is no substitute for sanitation and education with a broad "horizontal" base extending into the entire community. This is certainly not meant to imply that adequate sanitation within a compound such as Ludlow's should not be encouraged and even further improved; indeed, except for good sanitation, conditions within the densely populated labor lines might well be considerably worse than in the unsanitated but less crowded village environments. The fullest benefits of compound sanitation, however, cannot be fully enjoyed or exploited until basic sanitation and its corollaries become a reality beyond the compound walls as well as within them.

SUMMARY

A survey of intestinal parasites was conducted among 750 workers at an American-owned jute mill near Calcutta from March through June 1952. Infections were found in 92.5 per cent of the sample, 73.5 per cent with protozoan infections and 76.1 per cent with helminthic infections. The mean number of infections per person was 2.5, but nearly 10 per cent of the persons proved to have 5 or more (up to 8) infections.

The rate of infection with those parasites generally considered to be pathogenic follow in order of prevalence: hookworm (68.7 per cent), *Trichuris trichiura* (16.4 per cent), *Ascaris lumbricoides* (9.6 per cent), *Giardia lamblia* (9.1 per cent), *Entamoeba histolytica* (8.3 per cent), and *Strongyloides stercoralis* (8.1 per cent).

Slight general differences were noted in comparing rates for different age groups, males and females, and Hindus and Muslims. The most striking variance, however, concerned the much higher prevalence of ascariasis and trichuriasis among Muslims (29.9 and 46.7 per cent respectively) as compared with Hindus (3.0 and 6.5 per cent respectively).

No differences with respect to parasite prevalence were found between workers living in sanitated quarters within the mill compound as compared with those living in the generally unsanitated environment of the nearby villages. The significance of this observation is discussed and the limitations of "pin-point" sanitation are indicated.

Acknowledgements

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PROBLEMS IN TROPICAL PUBLIC HEALTH AMONG WORKERS AT A JUTE MILL NEAR CALCUTTA

III. Intestinal Parasites in the European Supervisory Staff and their Foodhandler Servants

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As a corollary of a survey of intestinal parasites among 750 workers at the Ludlow Jute Co. mill at Chengail (Howrah District, West Bengal), a simultaneous study of a similar nature was conducted among the members of the mill's "European" supervisory staff, their families, and their foodhandler servants. The results of the latter survey are embodied in the present report for, despite their small number, the health of the supervisory group is obviously as important to the proper functioning of industry in the tropics as is that of the labor force as a whole. Apart from this consideration, it is also of interest to compare the results of the survey among the workers (Chernin, 1954) with those presented below for their supervisors, bearing in mind the vast differences in living conditions, habits and privileges that separate the two groups.

MATERIALS AND METHODS

Clean, covered tins were distributed to all members of the supervisory staff with the request that the stools be passed in them and brought to the laboratory within one-half hour of their passage. A few stools were not delivered within that time period but in no case was the known interval from passage to examination in excess of one hour. The stools of the foodhandler servants were collected at the laboratory in the same manner that was employed during the mass survey of the mill workers (Chernin, *loc. cit.*) and the stools were examined within 20 minutes after passage. The same technics of examination (one fecal smear in saline, one in Velat's stain, and one coverslip-collected zinc sulfate centrifugal concentration specimen) were applied to each stool in this study as had been employed in the mass survey, and records were maintained in a similar manner. All of the supervisors and the members of their families were examined between March 26 and July 2, 1952, while the survey among the servants was conducted between June 25 and July 4, 1952. Only one stool from each person was examined.

COMPOSITION OF THE SAMPLE GROUPS

1. *"European" supervisory staff and families.* This group includes a total of 62 men, women and children, and is, despite its designation as "European," actually one-third Indian in its composition. The Indians are, however, of the same general socio-economic class as their Western counterparts and all live under identical conditions at the Chengail mill. The detailed composition of this group by country of origin is as follows: India, 19 (31 per cent); U.S.A., 13 (21

per cent); Ireland, 12 (19 per cent); Scotland, 9 (14.5 per cent); and England, 9 (14.5 per cent). Thirty-nine were males, 23 were females and 9 were children below 10 years of age. The adults ranged from 18 through 62 years of age with 44 per cent of the total in the age group 30–39, and 25 per cent in the 20–29 year group. Eleven (18 per cent) had lived in the tropics less than 1 year, 18 (29 per cent) for 2–4 years, 8 (13 per cent) for 5–9 years, 6 (10 per cent) for 10–12 years, and 19 (30 per cent) had lived in the tropics for 20 years or longer. Only a few individuals had ever resided in tropical countries other than India.

2. *The foodhandler servants.* This heterogeneous group of 47 includes all of the cooks, bearers, and baby *ayahs* in the service of the supervisory households; excluded from the group are the sweepers, who, by the nature of their work, have no functional contact with the food consumed in the households. Of the 47 (39 males and 8 females), 95 per cent were between 20 and 49 years of age, and the balance 50 years or older. The largest proportion (28 per cent) were natives of West Bengal, and the remainder came from East Pakistan (23 per cent), Orissa (19 per cent), Bihar (17 per cent), and Madras (6 per cent); persons from the United Provinces, Burma and Nepal together constituted the remaining 6 per cent of the group. Most of the servants were Muslims (54 per cent), while 35 per cent were Hindus and 11 per cent were Christians. The periods of service in various households at the mill ranged from less than one year to a maximum of 25 years.

SURVEY RESULTS

The results of the survey of intestinal parasitic infections in the two groups under consideration are summarized in Table 1. Protozoan infections were found in 39 per cent of the staff personnel and in 83 per cent of their servants and the differences between the two groups with respect to the prevalence rates of the various intestinal protozoa is evident in the tabulation in most cases. It is of particular interest that *Entamoeba histolytica* was equally prevalent in both groups and that the rates are of the same order as the rate previously established (8.3 per cent) for the 750 mill workers (Chernin, *loc. cit.*). With respect to helminthic infections there is a wide difference between the supervisory group and their servants; only 1 of the 62 staff persons had a helminthic infection while nearly 80 per cent of the servants were worm-infected. In all, 40 per cent of the supervisors and 87 per cent of their servants proved to be infected with one or more intestinal parasites.

Among the supervisors and dependents from the United States, 54 per cent had intestinal parasites, followed by those from Scotland (44 per cent), India (42 per cent), England (33 per cent), and Ireland (25 per cent). Those who had been in the tropics 5–9 years had the highest rate of infection (63 per cent) while those who had resided one year or less in the tropics were only 27 per cent infected. Among the servants, Hindus were 75 per cent infected, Christians 80 per cent infected, and Muslims 96 per cent infected; 63 per cent of the females and 92 per cent of the males had one or more infections.

In addition to the differences between the supervisory group and the servants

TABLE 1

General summary of the prevalence of intestinal protozoan and helminthic infections among the supervisory personnel and their families (62 persons) and among their foodhandler servants (47 persons) at the Chengail mill of the Ludlow Jute Company

INTESTINAL PARASITES	SUPERVISORY STAFF (TOTAL 62) INFECTED		SERVANT FOOD HANDLERS (TOTAL 47) INFECTED	
	Number	Per cent	Number	Per cent
Entamoeba histolytica	4	7	3	6
Entamoeba coli	10	16	20	43
Endolimax nana	4	7	9	19
Dientamoeba fragilis	1	2	4	9
Iodamoeba williamsi	0	—	1	2
Giardia lamblia	7	11	2	4
Trichomonas hominis	3	5	5	11
Chilomastix mesnili	0	—	0	—
Enteromonas hominis	0	—	0	—
Hookworm sp.*	0	—	23	49
Ascaris lumbricoides	0	—	8	17
Trichuris trichiura	1	2	8	17
Enterobius vermicularis	0	—	3	6
Strongyloides stercoralis	0	—	2	4
Hymenolepis nana	0	—	1	2
Total with protozoan infections	24	39	39	83
Total with helminthic infections	1	2	37	79
Total prevalence—all parasites	25	40	41	87
Mean no. infections per person	0.5		1.9	

* Species not determined.

TABLE 2

The proportion of persons carrying given numbers of intestinal parasite infections among the supervisory group as compared with their servants

NUMBER OF INFECTIONS	SUPERVISORY STAFF		SERVANT FOODHANDLERS	
	Number of persons	Per cent	Number of persons	Per cent
0	37	59	6	13
1	21	34	13	28
2	3	5	16	34
3	1	2	5	10
4	0	—	6	13
5	0	—	1	2
Totals	62	100	47	100

noted above in the prevalence rates, differences of a similar order of magnitude exist with respect to the proportions of persons carrying given numbers of infections (Table 2). While only 7 per cent of the supervisors had 2 or more infections (maximum of 3), nearly 60 per cent of their servants had 2 or more infections (maximum of 5). The mean number of infections per person in the former group was 0.5 per cent and in the latter 1.9 (Table 1).

DISCUSSION

The supervisory staff and their families occupy screened, spacious quarters in a series of large permanent buildings facing the Hooghly River. Each family unit is provided with modern bathroom-lavatory equipment. The water used for washing and bathing is the same filtered, chlorinated water which is supplied to the workers for drinking purposes (Chernin, *loc. cit.*); however, the drinking water used by the supervisory staff is first distilled in the mill's power plant and then delivered as needed to each household in glass carboys. Kitchens are equipped with a full range of modern appliances including electric refrigerators and all bedroom units are air-conditioned. Most vegetables consumed in the households are grown under supervision in a company-operated garden within the compound; other foods are purchased by householders in Calcutta as required. Household cooks are usually under careful supervision while at work and staff personnel visiting in Calcutta ordinarily restrict their dining to the more reputable restaurants.

Most of the servants live in quarters adjacent to the staff houses under conditions that very closely approximate those previously described for the mill workers' "lines" (Chernin, *loc. cit.*), although a few live in nearby villages. Many of the servants are literate, wear shoes, enjoy a better and more diversified diet than do the mill workers, and, on the whole, are somewhat more aware of basic sanitary concepts than are the ordinary mill-hands. Considering all these factors, it is not surprising that the servants as a group occupy a position somewhere between the supervisory staff and the mill workers as regards the prevalence of parasitic infections among them. The data presented also illustrate that, under the circumstances described, it is possible for Westerners to live in a tropical country with no great risk of intestinal parasite infections.

Two additional observations that are of interest may be noted briefly. None of the four cases of *E. histolytica* infections that were uncovered among the supervisory personnel occurred in a household in which any of the foodhandlers had the infection. Two of these cases presented symptoms attributable to the infection while the other two were essentially symptomless. The second point of interest is that seven of the eight cases of ascariasis among the servants, and an identical proportion of the trichuriasis infections, occurred in Muslims. This observation serves, within the limitations imposed by the small numbers involved, to substantiate the observation first made among the mill workers that these two infections are much more prevalent among Muslims than Hindus (Chernin, *loc. cit.*).

SUMMARY

A survey of intestinal parasites was conducted among the members of the supervisory staff, their families, and their foodhandler servants at the mill of the Ludlow Jute Co., Chengail, Howrah District, West Bengal.

In the supervisory group of 62 persons, 40 per cent were found to have intestinal parasites, 39 per cent with protozoa and 2 per cent with helminths. Among the servants (47 persons), 87 per cent were infected with some intestinal parasite, 83 per cent with protozoa, and 79 per cent with helminths. The mean number of infections per person among the servants was 1.9, four times the mean for the supervisory group.

Entamoeba histolytica was equally prevalent in both the supervisors and their servants (7 and 6 per cent respectively), and this rate compares well with the one derived earlier (8.3 per cent) from a survey of 750 of the mill workers. As in the latter survey, ascariasis and trichuriasis were found to be far commoner among Muslims than Hindus.

In general, the servants as a group fall between the supervisory personnel and the mill workers at large in terms of the prevalence of intestinal parasites among them. It is noted that under the conditions of living at the Chengail mill the risk to the supervisory group of acquiring intestinal parasite infections is no greater than it is generally assumed to be in their native Western countries.

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SPARGANOSIS IN KOREA

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The first description of a sparganum from man was based on Manson's discovery in Amoy, China, in 1881 of several large larvae in an autopsy subject (Cobbold, 1883). A number of specimens were found lying under the peritoneum in the neighborhood of the kidneys and iliac fossae, and one, apparently a free organism, in the pleural cavity. At about the same time, in Kyoto, Japan, Scheube recovered a sparganum from the urethra of a man suffering from a urinary tract infection (Leuckart, 1886). Since that time a number of other human cases have been reported. The following references serve as some interesting examples.

Most human infections have been encountered in the Orient. Miyake (1904) summarized 18 cases from Japan and added two new ones. In Korea, Kobayashi in 1925 reported that 3 human cases of sparganosis had been encountered. One was discovered by Uemura involving the leg of a Korean in Seoul and was described by Doi and Bok (1924); another from the lid of a Korean in Heijo was described by Kikkawa in 1924; and a very doubtful and unpublished case was reported from Nankai, South Keisho-do. In 1933, Kobayashi summarized information concerning human parasites in Korea and stated in regard to sparganosis that "... there are about 10 cases of infection of the human body.... In Korea, in the south, it is found on the islands in the Keisho-hokudo area and various other places. We cannot say for sure, but many more cases may exist." Miyazaki (1941) recently described a case of sparganosis in a male whose name is of Korean origin, but no further information is available.

During a nine year period in the Foochow area in China, Campbell, Webster and Li (1936) described 5 cases of sparganosis. In French Indo-China, various workers have contributed new information to the biology of sparganosis and have pictured in considerable detail the peculiar ocular infections that occur in that region (Joyeux and Houdemer, 1928; Houdemer, Dodero and Cornet, 1933; 1934; Joyeux, Truong and Nguyen, 1939; Galliard, 1948). In recent years, sparganosis infections presenting interesting pathology have been reported by Bonne (1930; 1942), and Bonne and Lie Kian Joe (1940) from Indonesia. Cleland (1918) referred to two previous cases in Australia and described a third. The first American infection was reported by Moore (1915) from a resident of Texas. Sambon (1907) has given a brief account of a sparganum extracted from the thigh of a Masai in British Central Africa.

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CASE REPORTS

Three cases of sparganosis in North Korean prisoners of war were encountered during the Fall of 1951 at the 64th Field Hospital on Koje-do.

Case 1. The patient, K. M. D., was a 39-year old male who had been a farmer in civilian life, living near Seoul. He was admitted to the hospital in September because of a swelling in the wall of the abdomen. About 40 days prior to admission, the patient noted the appearance of a mass the size of a fingertip in the right, midabdominal region, which was tender and produced an itching sensation. It gradually enlarged in a linear fashion downward and medially. The itching increased, but remained localized to the limits of the mass. The patient had the sensation of something moving and wriggling within the swelling. About two weeks after the mass appeared, he noted the onset of a second tender swelling, also about the size of a fingertip, in the right paraumbilical region. This one, however, remained stationary in size.

These two masses were noted when the patient was admitted to the hospital. The one in the right midabdomen was cylindrical, slightly oblique and almost in the position of a high McBurney incision. It measured about 8 to 10 cm. in length, about 2.5 cm. in diameter, and was in the abdominal wall. The mass in the right paraumbilical region was still the size of a fingertip and also in the abdominal wall.

On surgical exploration, the linear mass was found to consist of edematous and indurated internal oblique muscle. From this area of involvement a worm was withdrawn. No obvious capsule formation was noted around it when it was separated from the tissue. The paraumbilical mass yielded no worm on exploration, but here, too, the muscle was edematous and indurated.

Before fixation, the worm was approximately 30 cm. in length and 3 mm. in width. Unfortunately, additional observations were not made on the living organism, but it was fixed immediately in formalin. The worm (Fig. 1) was a typical sparganum: ribbon-shaped, showing pseudosegmentation and pale milk-white in color. The anterior end contracted considerably forming a subspherical expansion with a slight indentation at the anterior midline where the scolex was invaginated. The posterior end was flat and irregularly shaped.

The patient gave a history of eating raw flesh on several occasions; raw beef in August, 1950 and raw snake in April, 1951. He had also eaten raw river fish at various times during the past three years.

Case 2. The patient, J. T. W., was a 31-year old male from northeast Korea who was an unskilled laborer in civilian life. He was admitted to the hospital in September, a week prior to Case 1, also with the complaint of a swelling in the abdominal wall.

About three months before admission, the patient noted the appearance of a tender mass in the left upper quadrant of the abdomen. The mass fluctuated in size from that of a fingertip to that of a hen's egg. The tenderness was constant, as was the location. This patient also had the sensation of a wriggling object within the mass. No itching, however, was felt.

On admission to the hospital, the tender mass was noted. It was located in the left upper quadrant of the abdomen and was within the structure of the abdominal wall. The swelling was oval in shape and measured about 3.5 by 2 cm.

Because the history was so similar to that of the first case, it was anticipated that this might also be a sparganum infection. On surgical exploration, the mass was found to be an edematous and indurated area of external oblique muscle, and on dissection a sparganum was quickly located. Portions of the dissected muscle showed a grayish-green discoloration, but no obvious capsule was evident around the worm. The organism extended irregularly between the muscle bundles but was freed by dissection without too much difficulty (Fig. 5, area of surgical involvement). No motion of the worm was discerned during this period; it was approximately 25 cm. in length by 2 to 3 mm. in width. When placed in warm saline, it immediately became extremely active and motion pictures of its movements were made. Most of the activity consisted of rapid extensile and retractile movements of the anterior quarter to half of the organism, which were "probing" in nature. The posterior portion generally dragged along, or showed slow peristaltic waves which proceeded down the length of the worm. This portion appeared like a twisted ribbon, slowly elongating and contracting. The entire worm contracted and thickened after removal from the muscle to saline, and at this time varied from about 7 to 15 cm. in length. The anterior end of the organism was extremely thin and active when extended. The extreme tip, however, remained invaginated most of the time. Partial evagination occurred, but despite the fact that the worm was observed for a period of several hours, the bothria were never clearly revealed. After about five hours in saline, the worm began to show degenerative changes and fragmented into two parts. This probably resulted from an injury to the cuticle which had been broken in a few places by the forceps which were used to dissect the worm from the muscle. When fixed in formalin, the two portions of the worm underwent considerable contraction (Fig. 2).

This patient had a history of having eaten raw snake, dog, and beef in the past year.

Case 3. The patient, J. M. M., was a 34-year old male from northeast Korea who was a farmer as a civilian. In May, 1951, he noticed the appearance of a peanut-sized mass in the lateral wall of the lower left chest. At this time there was a sensation of movement within the mass accompanied by piercing pain. From May to October the pain was continuous. After physical labor particularly, an area about 15 cm. in diameter around the mass would swell, and even on rest it took approximately 10 days to subside. During this period it was painful and tender to the touch. The skin above the mass often itched when it was not swollen.

The patient was admitted to the hospital in October, 1951, complaining of cough and a dull pain in the left chest. Previous to entering the hospital, the lower left side had been swollen for about 10 days. On physical examination, a soft sausage-shaped mass was palpable in the left midaxillary line extending

from approximately the ninth rib to two fingers' breadth below the costal margin. It was approximately 9 cm. in length by 2 cm. in width. The mass appeared to be somewhat movable in relation to the underlying tissue and overlying skin. The patient complained of the sensation of an object "crawling" within the swelling.

The history was similar to the two previous sparganosis cases, and the patient was operated upon.[4] On exposure and dissection of the external oblique, a portion of a worm suddenly herniated through a cut in the muscle. The tissue which surrounded the worm was edematous, but showed no other changes grossly. Gentle tugging would not free the organism and it was necessary to continue the dissection (Fig. 4). The worm appeared to be fairly firmly embedded by a thin, adherent, fibrous reaction which seemed to form a capsule around it. The freed portion supported on wet gauze showed slight, slow, peristaltic, twisting motions. The dissection was continued forward, involving the anterior serratus muscles through which the worm had penetrated. The last portion of the organism curled back on itself for 3 to 4 cm. When the worm was removed to saline it was found that it had been severed near the posterior end. At this time the entire organism was approximately 23 cm. in length and about 2 mm. in width. When contracted, it was approximately 3.5 mm. at the widest part. Only when the worm was placed in warm saline did vigorous motion begin, which was similar in all respects to that described for the sparganum recovered from Case 2. The worm was finally fixed in formalin (Fig. 3).

This patient also gave a history of eating raw snake, which will be discussed in greater detail below.

All three patients had an uneventful recovery following surgery.

PATHOLOGIC EXAMINATION

Tissue for pathologic examination was available only from Cases 2 and 3. Each consisted of a ragged piece of dark brown muscular tissue traversed by broad irregular bands of connective tissue. On multiple cross sections there appeared in the connective tissue a few small empty cavities of irregular shape and about 5 mm. in largest diameter.

Microscopically, the tissue from both cases presented the same basic lesion. The striated muscle tissue was traversed by broad bands of loose and rather cellular fibrous tissue heavily infiltrated by eosinophilic leucocytes, lymphocytes, plasma cells, and a few macrophages. In various fields the eosinophils made up from ¼ to ¾ of the inflammatory cells. In the attached fascia there was marked inflammatory infiltration, predominantly lymphocytic and located about small vessels. Scattered macrophages filled with hemosiderin pigment were also present. Within the broader bands of fibrous tissue were several irregular and collapsed cavities corresponding to those noted grossly (Fig. 13). They were typically lined, from within outward, first by a mat of granular fibrinoid material containing many eosinophilic leucocytes, next a thin layer of

[4] Surgical removal of this sparganum was performed by 1st Lt. Ronald S. Seaton.

relatively acellular fibrin, then a thicker layer of large radially arranged fibroblasts and epithelioid cells, and finally a broad zone of vascular granulation tissue heavily infiltrated with eosinophilic leucocytes and blending with the bands of connective tissue traversing the muscle (Fig. 14). For the most part the lumina of the cavities were empty, but occasionally they contained a small amount of serous fluid or blood possibly introduced during surgical excision. No viable or recognizable portions of the parasite were seen in sections.

The inflammatory reaction about the parasite in Case 2 seemed clinically to have been relatively less active, though of shorter duration. Correspondingly, the muscle external to the bands of scar tissue showed relatively little alteration. Only along the edge of the more massive bands of scar tissue did muscle fibers show atrophy, along with the formation of a moderate number of multinucleated strap cells indicative of direct injury and regeneration.

In Case 3, of six months apparent duration, about twice as long as Case 2, the reaction about the parasite was clinically more violent as evidenced by the larger swelling and the gross edema of the surrounding tissues. Microscopically the walls of the cavities were thinner and less distinctly laminated. In addition, the muscle showed in areas fairly heavy diffuse infiltration of eosinophils along preexisting tissue planes and between individual muscle fibers, spreading them apart. Associated with this inflammatory reaction were a few foci of acute coagulation necrosis of muscle, with interstitial edema, deposition of a delicate fibrin mesh and beginning invasion of the necrotic muscle fibers by eosinophils and a few neutrophils and macrophages (Fig. 15). Along the edge of the broad bands of scar tissue, however, the muscle showed only atrophy and the formation of a few multinucleated regenerating strap cells.

In Case 2 only, in which the encapsulation of the sparganum was thicker and denser, there were found a considerable number of crystals among the reacting eosinophils. These crystals, found singly or in loose radiating clusters, typically had the form of long slender spindles with almost straight sides, tapering from the center gradually to a sharp point on either end. They ranged in size from about 1 x 10 μ to very large ones 10 μ wide and more than 80 μ long, though rarely was the full length of the larger ones contained in one section. The average size of the majority was about 4 x 40 μ. A number of the larger ones showed a tendency to clean transverse fracture across the center (Fig. 16). They were best seen in sections stained by the Masson trichrome method in which the crystals were stained a clear deep red. In hematoxylin-eosin stained preparations they were less clearly seen as they took on the same general pink shade of the background tissue. In unstained preparations examined in water they could be seen as refractile glassy crystals without inner structure, and showing in the larger ones a very faint greenish brown tint. They exhibited no birefringence under polarized light, whether examined in stained sections or in unstained sections mounted in water.

They occurred in loose ill-defined groups in the tissue, characteristically at some distance from the cavities, where the granulation tissue was becoming converted into denser scar tissue. Here the contained eosinophils appeared less

well preserved and partially fused into irregular masses, with pyknosis and fragmentation of nuclei and rupture of cytoplasm with spilling out of eosinophil granules. Only very rare small crystals were found in fields where the eosinophils were individually well preserved. These crystals have the morphologic characteristics of Charcot-Leyden crystals.

DESCRIPTION OF PLEROCERCOIDS FROM *Dinodon rufozonatum* AND THEIR INFECTIVITY FOR GUINEA PIGS

The three patients were closely questioned concerning the possible routes by which the spargana might have been acquired. All denied having applied frog poultices to wounds, nor had they ever heard of such a practice, a procedure which is known to result in human sparganosis in China and French Indo-China (Campbell, Webster and Li, 1936; Houdemer, Dodero and Cornet, 1934).

It has been known for some time that "*Diphyllobothrium mansoni*" is present in Korea. The normal secondary intermediate host, here, as in other parts of the Orient, is probably a species of snake (Kobayashi, 1925).

In view of the fact that all three patients presented a history of having consumed raw snake either within or in the vicinity of the prison compounds, it appeared of interest to determine the possibility that such a practice might be the method by which their infections were acquired. It was not possible to devote much time to this pursuit, but a brief investigation indicated that a species of snake very common to the area was infected with plerocercoids, and that these larvae could grow considerably in size when introduced into a mammal.

A specimen of *Dinodon rufozonatum*[5] was caught in a rice paddy in the vicinity of the prison compounds and was necropsied. Many plerocercoids were found; most were free or encysted on the membranes covering various organs (esophagus, stomach, kidneys, intestine). One large larva was wrapped around the dorsal aorta. A heavy concentration of plerocercoids was deeply embedded in the musculature on either side of the vertebral column.

Many of the plerocercoids were only loosely adherent to the peritoneal membranes. These larvae were easily detached and showed no sign of a host tissue reaction around them. Most of these organisms were irregularly elongate, flattened, and showed slight pseudosegmentation (Fig. 7). When placed in warm saline all were extremely active, exhibiting rapid extension and retraction of the anterior end, accompanied by almost complete evagination of the scolex.

Other larvae showed varying degrees of encapsulation, the cysts ranging from thin, transparent investing membranes to tougher, opaque sacs which were somewhat yellow in color (Fig. 6). The larvae in the membranous cysts when dissected out were normal in appearance and active in warm saline. Those within the more organized and opaque cysts were always surrounded by a cheesy, amorphous material. Figure 8 shows a cross section of such a cyst. When organisms were dissected from these, they were invariably moribund or dead,

[5] We are indebted to Corporal Don Dimmock, who caught the snake and to Dr. Doris Cochran, Associate Curator, Division of Reptiles and Batrachians, U. S. National Museum, for its identification.

as judged by the fact that they showed very weak or no movement in warm saline.

The plerocercoids, in general, varied considerably in size, ranging from about 2 mm. in length to about 2.5 cm.; the average was about 0.5 cm. in length by approximately 0.8 mm. in width.

Three guinea pigs were infected by incising the shaved skin and inserting either one or two plerocercoids subcutaneously. Guinea pigs 1 and 2 had one and two plerocercoids respectively placed in the right upper quadrant of the abdomen. Guinea-pig 3 had two plerocercoids inserted in the right lumbar region. All incised areas healed rapidly, and only in one pig did a transient, palpable mass appear toward the end of the first week; it was at the site of the original incision and remained only two days.

The twelfth day after infection, guinea pig 2 was found moribund, apparently suffering from pneumonia. It was necropsied and two spargana were recovered. One was removed from the vicinity of the axilla of the right front leg. A thin layer of muscle covered it, the worm lying free, coiled in the fascia separating the muscle layers. The anterior end of the worm was contracted and thickened presenting a lanceolate appearance; the posterior portion was a thin flat ribbon. There was no gross evidence of tissue reaction to the larva which was easily removed. When placed in saline, there was immediate vigorous motion in no way different from that of the spargana removed from the human cases. When completely extended the worm was approximately 15 cm. in length and 1.5 mm. in width but underwent contraction when fixed in formalin (Fig. 9). The second larva was found coiled in the fascia between the muscle layers dorsal to the pelvis on the left side. The muscle tissue in the vicinity of the worm was somewhat ecchymotic. The larva, in appearance, was like the one just described, except that it was only about 10 cm. in length by approximately 1.5 mm. in width.

Guinea pig 3 died 40 days after infection, also apparently from pneumonia. At necropsy, two large spargana were recovered. The first lay just ventral and to the right of the trachea, extending irregularly across it, to penetrate between the neck muscles, finally curving dorsad around the scalenus muscle (Fig. 10). There was no gross tissue reaction to its presence. Portions of the worm at various intervals were knotted into a ball-like mass. When completely extended the worm measured approximately 36 cm. in length by 2 mm. in breadth.

The second worm was found as an irregularly coiled and knotted ribbon (Fig. 11). At one end, it lay beneath the right pectoral muscle, then extended between the fascia of the serratus anterior. Just above the fifth rib, the worm penetrated deeper into another plane of the serratus, and then proceeded caudad to the level of the seventh rib where it ended bunched into a mass. The long, right thoracic nerve passed ventrally and was in contact with the worm. There were small areas of ecchymosis at the points of penetration through the different muscle layers, but other than that there appeared to be no reaction to the worm. The organism was not adherent at any point to the surrounding tissue, though it had to be dissected free because it was "sewn" through the muscle layers. In a

relaxed state it measured approximately 33 cm. in length by about 2 mm. in width. Both worms when placed in saline were very active, showing movements which were in no way different from that described for larvae recovered from the human cases. The spargana were fixed in formalin; one is shown in Figure 12.

Guinea pig 1 was necropsied on the forty-first day, but the larva with which it had been infected could not be found.

Only one guinea pig, (no. 2) which died on the twelfth day after inoculation, provided tissue suitable for histological examination. Though a portion of the cavity wall could be demonstrated, the sparganum had been present for too short a time to have provoked the multilayered thick encapsulation seen in the human tissues. Instead, sections showed a small portion of empty and collapsed cavity lined with fibrin mingled with partly necrotic leucocytes and red blood cells (Fig. 17). External to this there were a few macrophages, but no mantle of epithelioid cells or granulation tissue. For a short distance around the cavity the muscle fibers had undergone coagulation necrosis. The necrotic muscle fibers were being invaded and digested by monocytes and were spread apart by edema fluid and a moderate infiltration of monocytes and neutrophils but only rare eosinophils. Extending away from the cavity along the interstitial tissue planes was a moderate patchy or streaky infiltration composed of about equal numbers of monocytes, lymphocytes and neutrophils, and about 10 per cent eosinophils. No portion of the sparganum was seen in sections.

In addition to infecting the guinea pigs, some of the larvae recovered from the snake were placed in saline in a vial and shipped by air to the Laboratory of Tropical Diseases, National Institutes of Health, Bethesda, Maryland. Dr. Louis Olivier was kind enough to infect a dog with 4 plerocercoids which still showed some activity on arrival, in an attempt to determine the species. The feces of the dog were checked for eggs starting 10 days after infection, but by the thirtieth day none had been found, and the dog was necropsied. One small stunted worm about 2 inches long was recovered. It had a spatulate scolex with two bothria, and a neck to which was attached a string of small undifferentiated proglottids. Staining proved of no aid, and it was not possible to identify the organism as to species. Whether stunting had resulted from the fact that the larvae were moribund after their long journey from Korea or that the dog was not a suitable host is a matter of speculation.

DISCUSSION

The clinical symptoms which characterized the infection in all three humans were pain and swelling in the vicinity of the worm, a sensation of an object "crawling" within the inflamed mass and, in two instances, itching of the skin over the involved area. The masses had a tendency to fluctuate in size and recurrence of severe swelling in one instance was related to physical labor. Of interest in Case 1 was the finding of a worm in only one of the two swollen areas present. There is no proof that the swelling in the paraumbilical region from which no sparganum was recovered, had at one time contained one. From

the knowledge, however, of the migratory habits of these larvae, it is possible that they may elicit an inflammatory response, which may remain for a time to mark the site of an already departed organism. Ijima and Murata (1888) described a transient swelling in the right thigh of a Japanese soldier which would disappear for a period of time, only to reappear again in another area of the thigh. This occurred many times during a period of 9 years; a sparganum was eventually extracted from the swollen area.

In the pathogenesis of the tissue lesions in both man and the guinea pig, one of the unresolved problems is the precise manner in which the sparganum produces the tissue injury which is followed by so florid an inflammatory reaction. Several factors may be operative to bring this about. Excretory products which arise from the metabolism of the worm undoubtedly diffuse through its cuticle into the surrounding tissue and these may have a directly toxic action. This is supported by the fact that in the more acute lesions in man and guinea pig as shown in Figures 16 and 17, there was visible focal necrosis of muscle tissue. The possibility also exists that, in addition, the reaction may in part be allergic and may be due to sensitization to some of the products of the worm. Of interest in this regard are the clinical histories of transient swelling and edema associated in some individuals with marked itching at the site of the worm, as well as the rapid swelling and tenderness of the tissues surrounding the worm, which followed physical labor in Case 3. Histologically, although the intense eosinophilic infiltration of the tissues in the immediate vicinity of the spargana is not necessarily pathognomonic of an allergic inflammation, it is suggestive of one. Mueller and Chapman (1937) have shown also that in infections of monkeys with *Sparganum mansonoides*, complement-fixing antibodies are formed, and Mueller (1938) has demonstrated that the injection of powdered adults of *S. mansonoides* into monkeys infected with spargana of this species resulted in the localization and encapsulation of the living worms.

The finding of Charcot-Leyden crystals in the tissue of Case 2 is deserving of comment. Their occurrence, chiefly in areas containing degenerating eosinophils, is an interesting corroboration under natural conditions of the *in vitro* production of Charcot-Leyden crystals from the nuclei of eosinophils by treatment with detergents as described by Ayres and Starkey (1950). The absence of birefringence may possibly be the result of the dehydration of the tissues in the process of embedding, as Ayres and Starkey stated that their artifically produced crystals lost their birefringence when permitted to dry.

Houdemer, Dodero and Cornet (1934), in discussing the two innermost layers of the host reaction surrounding the spargana encountered in their human cases, refer to the presence of brown, diamond-shaped crystals of different sizes which, they felt, seem to be products of disintegration of cellular elements, and can be made up either of crystals of hematoidin or of Charcot crystals. Mueller (1940) has also described crystals which he believed to be those of Charcot-Leyden scattered among the cellular elements, particularly the macrophages, in the host reaction of man and monkeys infected with spargana. They occurred most abundantly after the infection reached a chronic state and the worms had become well walled off.

The guinea pig infections have several points of interest. Of particular note is the rapid rate of growth of the larvae. All plerocercoids which were used to infect guinea pigs were approximately 0.5 cm. in length. In guinea pig 2, which was necropsied 12 days after infection, the two worms which were recovered were 10 and 15 cm. in length respectively, averaging 12.5 cm., which gives an average increase of 12 cm. If, for the sake of simplicity, although it may not be absolutely correct, one assumes a uniform daily increment in length, then the average growth was approximately one cm. per day for these worms. In guinea pig 3, which was necropsied on the fortieth day after infection, the two larvae which were recovered measured 36 and 33 cm. respectively, averaging 34.5 cm. The average daily increase in length is close to what was found for the first two worms, being about 0.8 cm. There does not appear to be much information in the literature on the rate of growth of spargana. Kobayashi (1931) infected by mouth frogs, mice, white rats, cats, man and monkeys with procercoids which averaged 0.25 mm. in length. Unfortunately, in some instances the figures given in the text do not agree with those in the tables. However, growth of larvae in the warm-blooded hosts was more rapid than in the cold-blooded. In the frog at 35 days the plerocercoids averaged 2.8 cm. in length; in rats for the same period of time, 10.1 cm. At 40 days in the cat the plerocercoids averaged 9.8 cm.; at 90 to 98 days in the monkey, 12.5 cm.; and after 20 days in man one worm measured 1.5 cm. in length.

THE PRACTICE OF EATING SNAKE AMONG KOREANS AND ITS POSSIBLE RELATION TO SPARGANOSIS

As indicated previously, all three patients had a history of having eaten raw snake. Case 3 may serve as an example of this practice. He related that it was a common belief among Koreans that snake had special nutritive value which was particularly effective in combatting illness. For a number of years previous to his capture he had suffered from malaria and "typhoid" fever. In March, 1951, while in the prison compound, he caught and ate a snake about two feet long and one inch in diameter at a time when he feared his previous symptoms were reappearing. The snake's head was cut off, the skin stripped from the body, and the entire carcass including the viscera was then consumed raw. This was the first snake he had ever eaten. While outside the compound on a work detail a month later, he found another snake which was much bigger than the first and of which he ate half in the same manner as before. It is of interest to note that the first appearance of the tender swelling in the abdominal wall occurred approximately two months after he had eaten the first snake.

Further information on the practice of eating snake was obtained on interviewing 350 Korean patients at random in the hospital wards. Of these, 41 stated that at one time or another, generally previous to capture, they had eaten snake. Fourteen had eaten it raw, 25 had consumed it only boiled or roasted and two had eaten snake both raw or cooked. One preferred snake embryos. Most of these individuals did not eat snake consistently, but would only do so during a period of illness for the nutritional and medicinal effect. A few, however, relished snake as a delicacy, one having eaten about thirty during the previous year

alone. Although some gave histories of transient, tender swellings which appeared on various parts of the body highly suggestive of sparganosis, there was no way of determining conclusively from these histories alone whether any of these individuals was actually infected.

Human sparganosis can probably be acquired in several ways, by the ingestion in drinking water of *Cyclops* infected with procercoids as well as by the penetration of the skin by the procercoids (Yokogawa and Kobayashi, 1930), by the application to wounds of frog poultices containing plerocercoids (Houdemer, Dodero and Cornet, 1934; Campbell, Webster and Li, 1936), and by the ingestion of plerocercoids contained in the raw or partially cooked flesh of any of the secondary intermediate hosts.

Evidence supporting the last hypothesis has gradually accumulated over the past 30 years. Okumura (1919) obtained adult worms of *D. mansoni* in the dog by feeding plerocercoids from the snake, *Elaphe climacophora*, demonstrating the snake to be a suitable secondary intermediate host. He also found that plerocercoids from frogs and snakes inserted into the abdominal cavity of rabbits, guinea pigs, rats, mice and frogs were subsequently recovered alive, most of them having wandered into the muscles and subcutaneous tissues. Yokogawa and Kobayashi (1930) postulated that man could be infected by eating raw any of the secondary intermediate hosts of *D. mansoni* infected with plerocercoids. They presumed this on the sole ground that the majority of the human cases of sparganosis in Japan were middle-aged men who had occasionally taken raw or half-cooked chickens, which might have been infected with spargana. This method of infection was conclusively demonstrated when Yokogawa fed 3 spargana each, obtained from frogs, cats and the so-called Shyuso respectively, to three volunteers. He operated on one of the three subjects six weeks from the time of infection and found one sparganum from the frogs in the subcutaneous tissues of the left abdominal wall of the subject, while the other cases were found still negative in 3 to 5 weeks' observation, though the spargana may possibly have existed in some other unexamined internal portion of their bodies.

Iwata (1933) took plerocercoids from snakes and gave them to the monkey, *Macaca cynomolga*. Worms were later recovered from the hypodermis and muscle. The supposition on theoretical grounds that human sparganum infection may occur from the ingestion of uncooked flesh of frogs which may act as secondary intermediate hosts has also been suggested by Houdemer, Dodero and Cornet (1933, 1934) in French Indo-China, and Bonne (1937, 1942) in Indonesia.

That snakes parasitized by plerocercoids are present in Korea has been well established. Kobayashi (1925) found the reptiles *Natrix tigrina*, *Elaphe schrenckii* and *Agkistrodon blomhoffii* infected in Keiko-do, while Doi (unpublished) discovered the parasite in snakes in Heijo. Kobayashi stated that, peculiarly enough, he did not find frogs infected.

The evidence in the present report indicates that the three human cases described as suffering from sparganosis, ate raw snake; that a snake caught in the area was heavily parasitized with plerocercoids; and that these plerocercoids migrated and grew to great size when introduced into guinea pigs. It is sug-

gested that the practice of eating raw or partially cooked snake is a way by which sparganum infections are acquired by man in Korea, and that in view of the fairly widespread habit of eating snake, such infections in Korea may be more common than has been generally suspected.

The specific identity of the larvae dealt with in the present report is of course not known. The tendency is to consider the spargana involved in human infections in the Sino-Japanese area as the larvae of "*Diphyllobothrium mansoni*," but identification at the present stage of our knowledge must be based on the characteristics of the adult reared in an appropriate host. Even with the adult stage, there is considerable dispute concerning the validity of the various species which have been erected. Wardle and McCleod (1952) have discussed this problem in considerable detail and suggest that the genus *Spirometra* (Faust, Campbell and Kellogg, 1929), into which all these forms undoubtedly fall, be divided for convenience into two broad groups, the *bresslauei* group and the *okumurai* group. A great deal of additional experimental evidence will have to be accumulated before a truly satisfactory relationship can be established for the spargana encountered within the Orient, and elsewhere in the world.

SUMMARY

Three cases of sparganosis in Koreans were encountered. The larvae varied from 23 to 30 cm. in length and were removed by surgery from the musculature of the abdominal or lower chest area. The histopathology of the tissues in contact with the worms is described, and the presence of Charcot-Leyden crystals noted in one case in the masses of degenerating eosinophil leucocytes which preponderated in the inflammatory response. The pathogenesis of the tissue lesion appears to be due to a direct toxic effect of substances which probably diffuse through the cuticle of the sparganum, as well as a possible sensitization to some of these products. All patients gave a history of eating raw snake. A specimen of *Dinodon rufozonatum* caught in the area was found to be heavily parasitized with plerocercoids. When guinea pigs were infected with these by inserting the larvae subcutaneously, the worms migrated and grew at a rapid rate. It is probable that human sparganosis is acquired in Korea by the consumption of raw snake, which is a fairly common practice in this area.

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PLATES

Plate I

Fig. 1. Sparganum from human, Case 1; fixed in formalin
Fig. 2. Sparganum from human, Case 2; fixed in formalin
Fig. 3. Sparganum from human, Case 3; fixed in formalin
Fig. 4. Surgical removal of sparganum from Case 3
Fig. 5. Area of surgical involvement, Case 2
Fig. 6. Encysted plerocercoid on peritoneal membrane of *Dinodon rufozonatum*
Fig. 7. Unencysted plerocercoids removed from *D. rufozonatum;* fixed in formalin
Fig. 8. Cross section of encysted plerocercoid from *D. rufozonatum* showing thin outer capsule and larva embedded in mass of amorphous material; × 9.
Fig. 9. Sparganum removed from guinea pig 2, twelve days after infection
Fig. 10. Sparganum No. 1 *in situ*, guinea pig 3, forty days after infection
Fig. 11. Sparganum No. 2 *in situ*, guinea pig 3, forty days after infection
Fig. 12. Sparganum No. 2, from guinea pig 3; fixed in formalin

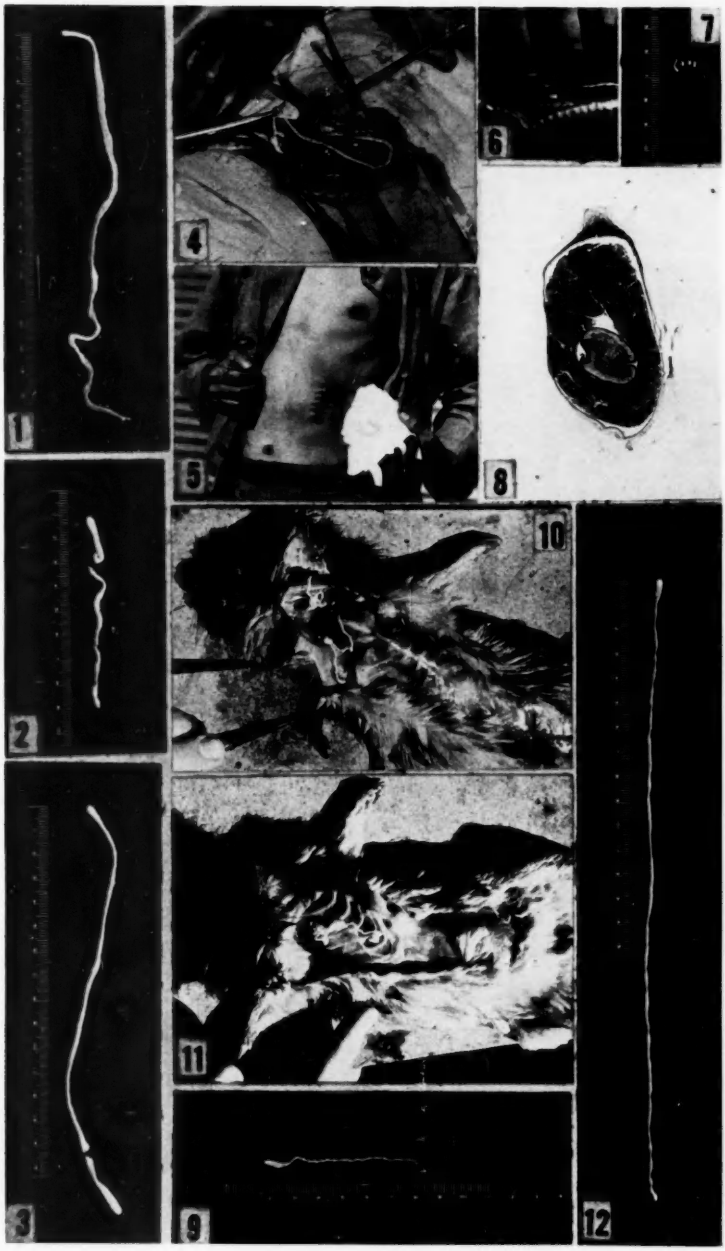
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Plate II

Fig. 13. Case 2. Cross section of the subcutaneous mass showing multiple cavities surrounded by broad bands of inflammatory tissue in the muscle; hematoxylin-eosin, × 4.

Fig. 14. Case 2. Section of laminated cavity wall showing, from within outward, layer of fibrinoid and eosinophils, layer of fibrin, layer of fibroblasts and epithelioid cells and broad zone of granulation tissue heavily infiltrated with eosinophils; hematoxylin-eosin, × 63.

Fig. 15. Case 3. Focal coagulation necrosis of muscle with inflammatory reaction and invasion of necrotic fibers by leucocytes; hematoxylin-eosin, × 235.

Fig. 16. Case 2. Crystals (Charcot-Leyden) among degenerating eosinophils in dense portion of granulation tissue; Masson Trichrome, × 945.

Fig. 17. Guinea pig 2. Margin of tissue in contact with sparganum, 12 days infection, showing thin layer of fibrin and leucocytes, and necrosis of adjacent muscle fibers; hematoxylin-eosin, × 112.

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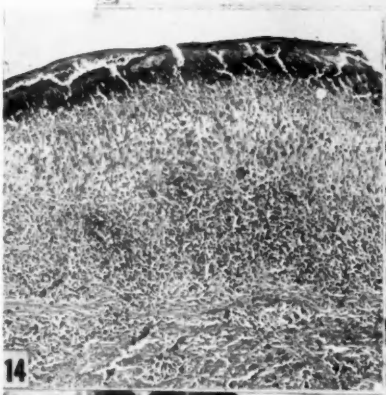

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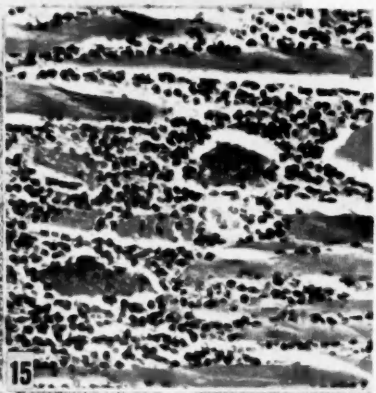

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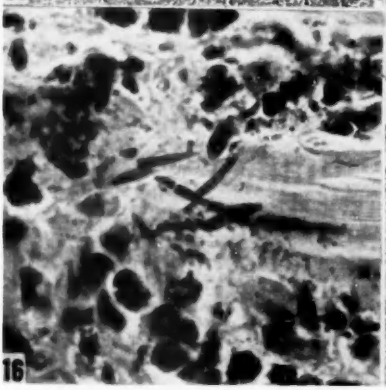

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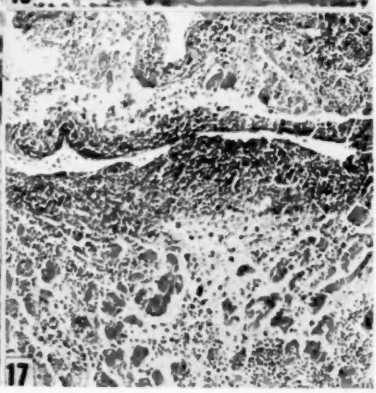

17

INTESTINAL PARASITES IN FOOD HANDLERS RETURNED FROM KOREA

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Korean veteran food handlers were considered a possible reservoir of parasitic disease when symptom-free intestinal parasite infections were incidentally discovered in Korean veterans under therapy for malaria. Subsequently, all Army personnel who had returned from Korea and were to be assigned as food handlers at Second Army Area installations were requested to submit a series of three stool specimens to be examined for parasites and eggs. This report presents the results of stool examinations performed on 287 food handlers returned from Korea and processed at Fort George G. Meade, Maryland, from January, 1952, through October, 1952.

MATERIALS AND METHODS

The specimens examined were collected at various dispensaries at Fort George G. Meade, Maryland, and forwarded to the Second Army Area Medical Laboratory, usually arriving on the day of collection. It was possible in most cases to examine the stools for the presence of eggs or parasites within several hours after the specimen had been collected. Three stools were submitted by most of the personnel and were obtained on three consecutive days. Forty-one of the group, however, submitted only one specimen each, while nine others submitted from six to fourteen specimens.

Direct saline and iodine (modified Dobell's) smears were prepared from only those specimens received the day of collection. Formalin-ether sedimentation (Ritchie, 1948) and acid-ether concentration (Weller and Dammin, 1945) methods were done on all stools submitted. The formalin-ether sedimentations were used to detect the protozoan cysts and infertile helminth eggs. The acid-ether method was performed to recover all other helminth eggs and larvae. Repetition of these technics plus water centrifugal sedimentation (Baroody and Most, 1946) were carried out on all stools which presented questionable findings on initial examination.

RESULTS

The 287 food handlers submitted a total of 795 stool specimens, an average of 2.8 stools per individual, for examination for parasites and eggs. One hundred and forty persons (48.8 per cent) were found to harbor parasitic infections. These infections were considered asymptomatic since no complaints were registered by the individuals examined, nor did dispensary medical officers

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TABLE 1

Incidence of intestinal parasites among Korean veteran food handlers

Total number of food handlers examined 287
Total number of stools examined (Av. 2.8 stools per food handler). 795
Food handlers positive for intestinal parasites 140 (48.8%)
Stools positive for intestinal parasites 312 (39.2%)

PARASITES FOUND	INDIVIDUALS	PER CENT	STOOLS	PER CENT
Endamoeba histolytica cysts	49	17.1%	74	9.3%
Endamoeba coli cysts	37	12.9	73	9.2
Endolimax nana cysts	100	34.8	175	22.0
Iodamoeba bütschlii cysts	9	3.1	16	2.0
Giardia lamblia cysts	12	4.2	35	4.4
Chilomastix mesnili cysts	1	0.3	2	0.7
Ascaris lumbricoides eggs	24	8.4	37	4.7
Hookworm sp. eggs	14	4.9	25	3.0
Trichocephalus trichiurus eggs	40	13.9	107	13.5
Hymenolepis nana eggs	1	0.3	4	1.4
Strongyloides stercoralis larvae	5	1.7	6	0.9

report physical findings indicating intestinal disorders. Table 1 shows the incidence of the parasites in the individuals and in the 312 positive stools submitted.

Many of the food handlers harbored multiple infections. The various infections harbored by the 140 parasitized individuals are shown in Table 2. Fifty-eight persons were found to harbor single species of parasites, 35 harbored two, 29 harbored three, 13 had four and 5 were positive for five species of parasites.

A total of 292 specific parasite infections was found. Forty-nine cases of *Endamoeba histolytica*, 12 cases of *Giardia lamblia*, and 84 cases of various helminth infections, a total of 145 possibly pathogenic infections, were discovered. Of these 145, 66 (45.5 per cent) were directly transmissible to recipients of food prepared by the food handlers (*E. histolytica*, *G. lamblia*, *Strongyloides stercoralis*). These 66 infections encompassed 62 of the 140 infected individuals. Thus, 21.6 per cent of all food handlers examined were carriers of directly transmissible pathogenic parasites.

DISCUSSION

These carriers represent a latent reservoir of disease and a potential threat that can have serious consequences in a military group. This fact has been recognized by Army authorities and it has become routine for all food handlers returning from Korea to submit stool specimens for examination for enteric pathogens before such individuals are assigned to kitchen duties.

The incidence of *Endamoeba histolytica* in this survey, 17.1 per cent of the total individuals examined, is not excessively high if compared with results of other surveys for amebic infection in food handlers. Rawlings and Fishbein in 1933 (Craig, 1944) reported an incidence of 23.6 per cent in their investigation, and Ritchie and Davis (1948) found 21.3 per cent of a group of Japanese food handlers to harbor *E. histolytica*. Similarly, our results fall well within the range

TABLE 2

Various parasitic infections found among 287 Korean veteran food handlers

	PARASITES FOUND	CASES
Single species	Endamoeba histolytica cysts	2
	Endamoeba coli cysts	7
	Endolimax nana cysts	25
	Giardia lamblia cysts	3
	Ascaris lumbricoides	5
	Hookworm sp. eggs	5
	Trichocephalus trichiurus eggs	9
	Strongyloides stercoralis larvae	2
Two species	E. histolytica, E. nana	12
	E. histolytica, T. trichiurus	1
	E. coli, E. nana	7
	E. nana, G. lamblia	2
	E. nana, I. bütschlii	1
	E. nana, A. lumbricoides	1
	E. nana, hookworm sp	1
	E. nana, T. trichiurus	6
	G. lamblia, A. lumbricoides	2
	A. lumbricoides, hookworm sp.	1
	A. lumbricoides, S. stercoralis	1
Three species	E. histolytica, E. coli, E. nana	9
	E. histolytica, E. nana, I. bütschlii	4
	E. histolytica, E. nana, A. lumbricoides	1
	E. histolytica, hookworm sp., T. trichiurus	1
	E. histolytica, E. nana, T. trichiurus	3
	E. coli, E. nana, T. trichiurus	1
	E. coli, E. nana, A. lumbricoides	1
	E. nana, A. lumbricoides, hookworm sp.	2
	E. nana, G. lamblia, T. trichiurus	2
	E. nana, A. lumbricoides, T. trichiurus	5
Four species	E. histolytica, E. coli, E. nana, I. bütschlii	2
	E. histolytica, E. coli, E. nana, T. trichiurus	4
	E. histolytica, E. coli, E. nana, A. lumbricoides	1
	E. histolytica, E. coli, A. lumbricoides, T. trichiurus	1
	E. histolytica, E. nana, G. lamblia, T. trichiurus	1
	E. histolytica, E. nana, T. trichiurus, S. stercoralis	1
	E. histolytica, E. nana, G. lamblia, C. mesnili	1
	E. histolytica, E. nana, E. coli, hookworm sp.	1
	E. nana, A. lumbricoides, hookworm sp., T. trichiurus	1
Five sp.	E. histolytica, E. coli, E. nana, I. bütschlii, H. nana	1
	E. histolytica, E. coli, E. nana, I. bütschlii, T. trichiurus	1
	E. histolytica, E. nana, G. lamblia, A. lumbricoides, T. trichiurus	1
	E. histolytica, E. nana, A. lumbricoides, hookworm sp., T. trichiurus	1
	E. coli, E. nana, hookworm sp., T. trichiurus, S. stercoralis	1
Total		140

of Faust's (1942) survey of the Western Hemisphere and Craig's (1944) summary for the Eastern Hemisphere. The 50 per cent amebic infection rate reported by Radke (1952) for a group of 93 Korean veterans is not approximated.

Had it been possible to obtain additional stool specimens from the 41 food handlers who contributed single specimens, a slightly greater number of *E. histolytica* infections would probably have been detected. The importance of multiple stool examinations was evidenced by the occurrence of *E. histolytica* cysts in only one of the three stool specimens submitted by several individuals. Frequently, it was necessary to perform additional emulsifications and sedimentations on larger portions of stool, particularly when *E. histolytica* had been diagnosed in the first of the series of three stools, but could not be found on initial examination of the second or third specimens.

In many cases the cysts of *E. histolytica* found were of the small race. Few cysts were found with chromatoid bars, but all other diagnostic characteristics were distinct. The pathogenicity of the small form of *E. histolytica* has been contested by Hoare (1952) and others. Hoare considers the organism to represent a commensal phase of *E. histolytica* found in symptomless carriers. Radke (1953) and D'Antoni (1952) and others, however, suggest that there are no symptomless carriers. In view of this difference of opinion, all *E. histolytica* infections encountered during this survey were considered potential, if not actual, threats to the health of the infected individuals and subsequently all were treated.

In surveys of this type, the incidence of *Endamoeba coli* usually exceeds that of *E. histolytica*. In this survey, however, the number of *E. coli* infections was nearly the same by stool findings, and there were fewer individual cases (37).

The *Endolimax nana* cysts observed were frequently of the large race. Often the cysts were found in large numbers. By virtue of the fact that the size of the large race *E. nana* cysts approximates that of the small race *E. histolytica* cysts, it would be possible for inexperienced technicians to fail to detect the latter when confronted with a microscopic field dense with *E. nana* cysts and with only a few *E. histolytica* cysts scattered among them. This size relationship required that the closest scrutiny be applied to each microscopic field when such mixed infections were encountered.

With few exceptions the helminth infections, as visualized by the recovered eggs, were light. The concentrations of eggs varied throughout the series of three stool specimens in most cases, but in only a few instances were the second or third specimens negative when eggs had been found in the first of the series.

A preponderance of the *Ascaris* eggs recovered were infertile, perhaps indicating a paucity of males in the *Ascaris lumbricoides* population within the host. The formalin-ether sedimentation method proved consistently efficient in concentrating these infertile eggs, while the acid-ether concentrations yielded few infertile *Ascaris* eggs.

The hookworm eggs recovered could not be identified conclusively as being those of either *Ancylostoma duodenale* or of *Necator americanus*, inasmuch as no adult worms were ever recovered from any stool collected during or following treatment. In addition, attempts to establish the parasite in experimental

animals by culture of the viable eggs and subsequent exposure to the filariform larvae were not successful. Measurements of these eggs indicated that both hookworm species were represented.

All of the food handlers found to harbor parasites, pathogenic or otherwise, were treated. These individuals were not assigned to duties in the post messes until a series of three negative stools had been submitted by each. For the most part, treatment was successful. Hookworm and whipworm infections, however, were highly refractory to treatment.

Had routine stool examinations not been conducted, these so-called "healthy carriers" would have gone undetected and would have been placed in positions which would theoretically have enabled them to transmit *E. histolytica*, *G. lamblia*, and *S. stercoralis* to susceptible persons. The frequency of apparently asymptomatic single and multiple parasitic infections in Korean veteran food handlers suggests that transmission hazards be given greater recognition.

SUMMARY

A survey for the presence of intestinal parasitic infections among 287 Korean veteran food handlers is presented. Results show that 140 (48.8 per cent) of these food handlers harbored, without apparent clinical manifestations, intestinal parasites varying from a single species to a mixed infection of as many as five species. The importance of routine multiple stool examinations is emphasized.

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THE PENETRATION-TIME FOR THE CERCARIAE OF *SCHISTOSOMA JAPONICUM*

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Although innumerable animal infections with *Schistosoma japonicum* have been performed since its life cycle was established (1909–1914), the time required for its cercariae to gain entry into the mammalian host has not been conclusively demonstrated. Faust (1949) mentioned that entry of schistosome cercariae into the epidermis may require less than 10 minutes. Iio (1928) investigated this problem with cercariae of *S. japonicum* by means of histopathologic sections, while Hitchcock (1949) directly observed penetration of the cercariae of *S. mansoni*. Currently, on the basis of worm recovery at autopsy, evidence of a more conclusive nature has been obtained regarding the penetration-time for the cercariae of *S. japonicum*. The rate of recovery of cercariae as adult worms was the basis of comparison for varied exposure intervals.

METHODS

The experimental animals (mice and hamsters) were clipped and shaved over most of the back 24 hours before exposure. The method of exposure was that previously reported from this department by Pan *et al.* (1951), with the exception that the animals were held by hand. Commonly, 75 to 100 cercariae were used for each infection. Variations in the time allowed for penetration varied from 10 seconds to 5 minutes. Immediately upon completion of exposure, the animals were thoroughly rubbed dry with a piece of gauze slightly moistened to enhance absorption. They were sacrificed three to four weeks later. The recovery of the adult *S. japonicum* was facilitated by the use of the perfusion technic previously described by Pan and Hunter (1951).

An attempt was also made to determine whether the water film and/or evaporation of water adhering to the skin after exposure enhanced penetration. This was done by using short exposure periods (10 and 15 seconds) followed by air-drying under an electric fan, which required less than five minutes, and comparison of the worm recovery with that of the three and five minute exposures which were terminated with immediate drying of the animals.

RESULTS AND DISCUSSION

A tabular representation of data is furnished in Table 1. Infections were obtained in mice with periods of exposure as short as 10 seconds, while for hamsters the shortest interval affording infections was 30 seconds. Although 50–75 per cent of the animals became infected for all exposure intervals under three minutes, the percentage of the cercariae recovered as adults at autopsy remained low and did not increase progressively, particularly in the mouse series. The few

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TABLE 1

Recovery rates of cercariae as adult schistosomes with different exposure times

EXPOSURE TIME	ANIMALS EXPOSED	ANIMALS INFECTED	PER CENT INFECTED	TOTAL CERCARIAE	WORMS RECOVERED	RECOVERY RATE
			Mice			
			(Animals were wiped dry immediately after exposure)			
						%
10 sec.	9	7	77.8	1086	44	4.1
15 sec.	10	5	50.0	1021	90	8.8
20 sec.	10	7	70.0	850	87	10.2
30 sec.	10	7	70.0	674	49	7.3
1 minute	9	5	55.6	394	13	3.3
2 minutes	4	3	75.0	199	13	6.5
3 minutes	14	13	92.9	727	378	52.0
5 minutes	10	10	100.0	446	246	55.2
			Hamsters			
			(Animals were wiped dry immediately after exposure)			
20 sec.	8	0	0.0	605	0	0.0
30 sec.	6	3	50.0	557	8	1.4
1 minute	8	4	50.0	697	57	8.2
2 minutes	5	5	100.0	482	68	14.2
3 minutes	3	3	100.0	176	172	97.7
			Mice			
			(Animals quickly air-dried (3–5 min.) by electric fan)			
10 sec.	10	10	100.0	1037	372	35.9
15 sec.	5	5	100.0	565	370	65.5

worms taken at autopsy for periods less than three minutes may represent those cercariae which were protected from wiping by gaining access to skin follicles or crevices, actual penetration of the epidermis occurring later.

When three minutes were allowed for penetration, a striking increase in the infection level occurred. For this interval, 97.7% of the cercariae applied to hamsters were recovered as adult worms. The number of hamsters used was small, but such results have, not uncommonly, been obtained in previous experiments where 30 minutes were allowed for penetration. When three-minute exposures were used for mice, 52.0% of the cercariae were recovered, while for five minutes this figure increased only to 55.2%. An explanation of this difference in recovery rate for mice and hamsters, such as might be obtained from histopathologic sections of skin and internal structures, is not at present available. Essentially the same results have been obtained consistently in this laboratory for mice with exposures of 30 minutes. It appears, under the conditions of the method used, that three minutes afforded near-maximum infections in both hamsters and mice.

These results are essentially in agreement with the work of Hitchcock (1949) who observed actual penetration. For 75 cercariae seen entering the skin of

hamsters, the average penetration time was 4.3 minutes, the range being 2–15 minutes. For mice, with 50 cercariae observed as penetrating, the corresponding figures were 3.6 minutes and 2.5–8 minutes.

Apparently, and fortunately so, the method here used for exposing the animals (Pan *et al.*, 1951) must tend to foster almost simultaneous contact with the skin by all cercariae. This is reflected by the abrupt occurrence of a near-maximum recovery of cercariae after three-minute exposures. For this procedure, an excess of three to five minutes time for infecting mice and hamsters is not warranted. This not only saves time, but also reduces experimental damage for the animals to a minimum. Although conclusions can be drawn only for the animals tested, the hazard of exposure for laboratory and field investigators may be inferred. Under natural conditions, such a rapid penetration by cercariae certainly increases their chance of completing the infection cycle.

Only limited observations were made on the effects of the water film and/or evaporation on penetration (Table 1). Combining short contacts (10 and 15 seconds) with rapid air-drying (total time not exceeding three–five minutes) recovery percentages of 35.9 and 65.5 per cent were obtained for mice. The effectiveness, or hazard, of even very short contacts with water containing cercariae, followed by air-drying, is evident. This is made possible by the tendency of the water film and attached cercariae to adhere to the skin, even with momentary contact. The recovery rates of 52.0 and 55.2 per cent (mice) for three and five minute exposures, terminated with immediate drying, were exceeded by the 15 second exposure combined with rapid air-drying (65.5 per cent). This suggests some benefit from the water film and/or evaporation, but the observation is inconclusive due to the small number of mice used for 15-second exposure. However, a method of determination may have been developed. Such an experiment is not indicated using hamsters, as maximum infections were obtained without air-drying.

SUMMARY

It has been demonstrated that near-maximum infections of *Schistosoma japonicum* can be achieved with three minute exposures terminated with immediate drying. For mice the recovery of cercariae as adults was 52.0 per cent, while for hamsters, 97.7 per cent. For all exposures under three minutes, only mimimal recovery of cercariae was achieved. Infections occurred in mice and hamsters after contact periods as short as 10 and 30 seconds, respectively, and such periods were highly effective when air-drying followed.

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OBSERVATIONS ON PORTAL HYPERTENSION AMONG SCHISTOSOMIASIS PATIENTS WITH RELATIVELY INSIGNIFICANT COMPLAINTS

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Chronic liver disease with portal hypertension is a familiar late sequela of schistosomiasis. The third and final clinical and pathologic stage of both schistosomiasis *mansoni* and *japonica* is dated from the time liver or portal venous system disease has progressed to the point of prominent secondary portal hypertensive changes. Pons (1937), in fact, stated: "We have been impressed from the very beginning by the frequency with which the intrahepatic portal circulation becomes seriously involved (in schistosomiasis *mansoni*), to the point, in fact, where the consequent hepatosplenic pathology and symptomatology dominate the pathologic and clinical pictures . . . so much is the hepatosplenic pathology a part of the disease caused by the *S. mansoni* that we can find no justification for its being termed 'intestinal schistosomiasis' . . ." Esophageal varices, massive hemorrhage and death are not uncommon (Almy and Harper, 1944; Bovaird and Cecil, 1914; Day, 1933; Faust and Meleney, 1924; Koppisch, 1932, 1941; Pons, 1937). The frequency of portal congestion as a late complication is indicated by Plaza Izquierdo's (1943) observation that in Venezuela Banti's syndrome is almost always due to schistosomiasis *mansoni*.

Schistosomiasis may be responsible for both of the mechanisms which ordinarily lead to portal hypertension: intrahepatic block and extrahepatic portal obstruction. The pathology of the former, or cirrhotic mechanism, has been well described (Faust and Meleney, 1924; Koppisch, 1941; Pons, 1937), is easy to comprehend and is familiar to those who have an interest in other forms of cirrhosis.

Mechanisms leading to extrahepatic portal block are not so well understood. Symmers (1951) found at autopsy areas of fibrosis and granuloma containing schistosome eggs in a branch of the portal vein close to the porta hepatis. Koppisch (1941), too, found examples of both splenic and portal vein fibrosis and pseudotubercle formation about esophageal varices. There was one instance of extensive portal vein calcification. Thrombosis of various segments of the portal system has been described (Faust and Meleney, 1924; Koppisch, 1941).

The history of the surgical attack on schistosomal portal hypertension parallels that which has been followed more actively for Laennec's cirrhosis. Omentopexy and mediastinal packing apparently have received only brief trial in this disease. Splenectomy has been a popular procedure, more for mere elimination of the splenic mass than for its rather negligible effects on the portal venous volume

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(Días, 1952; Plaza Izquierdo, 1943). Variceal sclerosis has been practised in schistosomiasis by Muñoz-MacCormick (1948) and may be considered adequate therapy in a few carefully selected patients. Portal decompression by venous shunt surgery has not yet been employed to any extent in this disease. Although end-to-side portacaval anastomosis is presumed to be the procedure of choice if conditions permit, splenogram might indicate the necessity for splenorenal shunt by revealing portal vein obstruction.

Therefore although production of portal hypertension and esophageal varices during the late stage of schistosomiasis is well known, it was believed that the potential danger of silent varices necessitated routine survey for this complication among patients whose disease was either early or largely subclinical. The present communication deals with a group of young soldiers with proven schistosomiasis. Observations on the nature of demonstrated varices, as well as experiences with portacaval anastomosis in their treatment, are presented. Although the subjects were not asymptomatic controls, they were young men who had within a few months or a couple of years passed a rigid Army physical examination and whose symptoms now were not those of portal hypertension.

MATERIAL AND METHODS

Forty-six active-duty soldiers with schistosomiasis were studied for signs of portal hypertension. Some had been brought up in endemic regions of Puerto Rico and some first came in contact with the infection during World War II, either in Leyte or Mindanao. In 14 the disease had previously been diagnosed and treated, but now stools, rectal aspirations, rectal biopsies, or all were positive for eggs. Table 1 categorizes the complaints which led the patients to seek medical help, and Table 2 outlines details regarding the schistosomiasis, methods of study and gross findings.

Eggs were found in 11 of the 20 livers which were biopsied, although two biopsies were necessary for their demonstration in two instances, and four biopsies were necessary in another (Fig. 1). Specimens from eight livers were considered to be normal. In brief, the liver pathology encountered was dominated by periportal fibrosis (Fig. 2), plus foreign-body (egg) granulomatosis.

One or more batteries of liver function tests were run in all 46 cases, and in 14

TABLE 1

Categorization of the symptom complexes which led to diagnosis

	S. japonicum	*S. mansoni*
No. patients	29	17
No symptoms (routine study)	8	1
Complaints due to other disease	0	2
Complex dyspepsia	4	1
Complaints ascribed to liver disease	5	8
Hematemesis the only complaint	2	2
Diarrhea	8	3
Fatigue, weakness	2	0

TABLE 2

Data on nature of infections, correlated with findings associated with portal hypertension

SPECIES	*S. mansoni*	*S. japonicum*	
Geographic source infection	Puerto Rico	Leyte	Mindanao
No. patients	17	3	26
Apparent duration infection:			
Range, years	15–22*	1½	4–10
Average, years	17.5*	1½	5¼
No. with ascites	0	0	0
No. with splenomegaly	11	0	3
No. with distended abdominal veins	0	0	0
No. gastroscoped	8	3	25
No. with gastric varices	0	1	0
No. esophagoscoped	17	1	3
No. with esophageal varices	6	1	0

* Because all of these patients grew up in endemic regions, the figures are derived arbitrarily by subtracting 6 years from each patient's age.

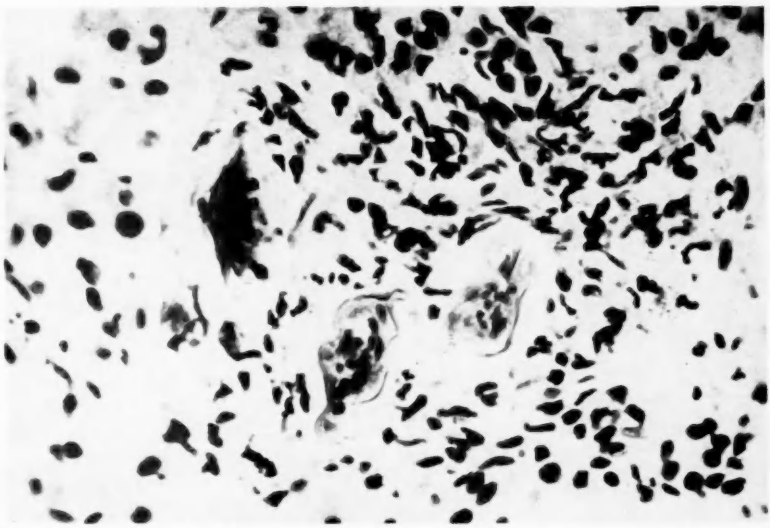

FIG. 1. Liver biopsy, showing three eggs with granulomatous reaction. Neighboring hepatic cells are normal.

there were significant abnormalities (Table 3). Elevation of serum bilirubin and increased bromsulfalein retention were the chief findings. There was no important abnormality among the flocculation tests, serum proteins, prothrombin concentration, or esterification of cholesterol.

The diagnosis of varices was made by esophagoscopic and gastroscopic examination, technics which at this Hospital have proved more efficient than radiologic ones (Brick and Palmer, in press). Portal pressure within esophageal varices

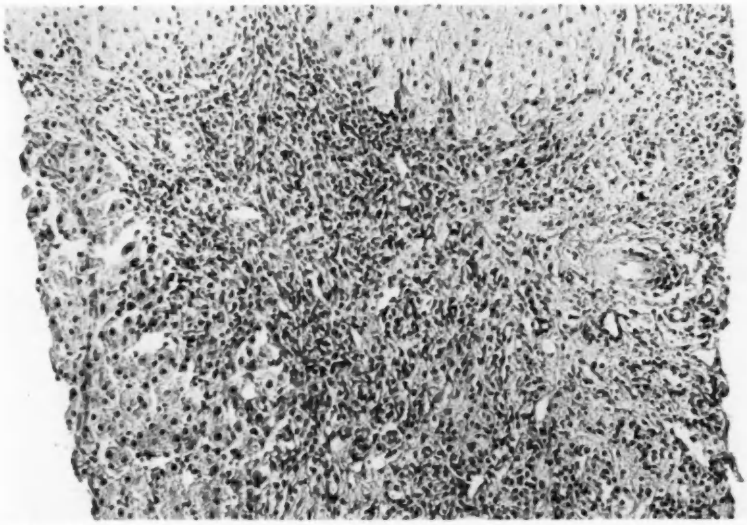

FIG. 2. Liver biopsy, showing area of severe fibrosis. In this field there are no eggs or granulomatous areas.

TABLE 3

Summary of abnormalities of liver function tests: admission studies, 14 patients (8 S. mansoni, 6 S. japonicum)

	NO. PATIENTS SHOWING ABNORMALITY	RANGE OF ABNORMALS
Bromsulfalein retention	12	8—24%
1 minute serum bilirubin	9	0.6–6.0
Indirect serum bilirubin	9	0.6–3.3
Serum albumin/globulin	3	To 2.7/4.4
Cephalin flocculation	3	3–4
Thymol turbidity	1	12
Prothrombin concentration	1	50%
Zinc sulfate turbidity	0	
Alkaline phosphatase	0	
Cholesterol esterification	0	

was measured transesophagoscopically by a technic previously described (Palmer, 1952).

FINDINGS

Upon clinical examination, evidences of portal hypertension were notably sparse, except for the incidence of splenomegaly among those with presumably chronic infections (Table 2). Nine of the patients gave a history of significant hemorrhage from the gastrointestinal tract, and four had no complaint other than sudden hematemesis (Table 1). Following radiographic and endoscopic

investigation of the upper and lower gastrointestinal tracts, it was concluded that esophageal varices had been responsible for hemorrhage in six instances.

Esophageal varices were diagnosed esophagoscopically in seven patients and gastric varices gastroscopically in one (Table 4). Radiographic study revealed esophageal varices in only one of these patients. In all cases, the most distal portion of the esophagus was involved by the largest varices, as is the case in portal hypertension secondary to Laennec's cirrhosis. The extent and severity (relative diameter) of the lesions were found to reflect to some extent the level of the portal venous pressure, as determined by transesophagoscopic pressure measurements. In this series the pressure levels corresponded to pressures measured within varices in moderate and severe cases of Laennec's cirrhosis (Palmer, Sborov and Jahnke, 1953).

As expected, specific therapy directed against schistosomiasis had no clear-cut effect on varices as judged esophagoscopically (Table 5). In one patient, moderate varices of the distal half of the organ had disappeared two months after the completion of therapy, but in three others there was no important change.

TABLE 4

Varices: data on the seven instances encountered

PATIENT; SPECIES; DURATION INFECTION (YEARS)	ESOPHAGEAL VARICES			GASTRIC VARICES	SURGICAL PROCEDURE
	Extent of involvement	Severity (diameter)	Portal pressure, mm. water		
1. S.j.: $1\frac{1}{2}$	Distal $\frac{1}{3}$	Mild	—	Yes	None
2. S.m.: 18	Distal $\frac{2}{3}$	Mod.	—	No*	Splenect.
3. S.m.: 18	Distal $\frac{2}{3}$	Sev.	430	No*	Portacav.
4. S.m.: 16	Distal $\frac{2}{3}$	Sev.	390	No*	Portacav.
5. S.m.: 17	Distal $\frac{1}{3}$	Mod.	—	No*	None
6. S.m.: 16	Distal $\frac{1}{3}$	Mod.	340	No	None
7. S.m.: 21	Distal $\frac{1}{3}$	Mod.	390	No	None

* Not gastroscoped; based on radiologic data only.

TABLE 5

Effect of specific schistosomiasis therapy on esophageal varices and portal venous pressure (mm. water)

PATIENT	TREATMENT: DRUG; TOTAL DOSE; COURSE	VARICES: PRETREATMENT. EXTENT; SEVERITY; PRESSURE; INTERVAL BEFORE NEXT EXAM.	VARICES: POST-TREATMENT. INTERVAL FOLLOWING TREATMENT: EXTENT; SEVERITY; PRESSURE; INTERVAL BEFORE NEXT EXAM.
1	tart. emet. 2.40 Gm., 32 days	$\frac{1}{3}$; mild; —; 10 days $\frac{1}{3}$; mild; —; 12 days $\frac{1}{3}$; mild; —; 5 weeks	2 days: $\frac{1}{3}$; mild; —; 5 weeks $\frac{1}{3}$; mod.; —
2	Fuadin 70 ml., 28 days	$\frac{1}{2}$; mod.; —; 3 mos.	8 weeks: no varices
6	Fuadin 90 ml., 20 days	$\frac{1}{3}$; mod.; 340; 4 mos.	13 wks.: $\frac{1}{3}$; mild; 310
7	Fuadin 90 ml., 21 days	$\frac{1}{3}$; mod.; 390; 4 mos.	14 wks.: $\frac{1}{3}$; mod.; 400

Three patients were treated surgically. In one simple splenectomy was done, more to control severe hypersplenic hematologic disease than to influence portal pressure. The extent of variceal involvement was found to be decreased to some extent three months later. Two patients were treated by end-to-side portacaval shunt (Fig. 3), a procedure discussed in detail elsewhere (Jahnke *et al.*, in press). During the operation the venous pressure measured in the portal vein fell from

FIG. 3. Anastomosis, following completion of portacaval shunt for schistosomal portal hypertension. The portal vein enters the operative field from above, to join the cava in an end-to-side anastomosis.

TABLE 6

Effect of surgical portal decompression on esophageal varices and portal venous pressure (mm. water). All patients previously treated for schistosomiasis

PATIENT	OPERATION	VARICES: PREOPERATIVE. EXTENT; SEVERITY; PRESSURE; INTERVAL BEFORE NEXT EXAM.	VARICES: POSTOPERATIVE. INTERVAL SINCE OPERATION: EXTENT; SEVERITY; PRESSURE; INTERVAL BEFORE NEXT EXAM.
2	Splenect.	$^2/_3$; mod.; —; $3^1/_2$ mos.	3 mos.: $^1/_2$; mod.; —
3	Portacav.	$^2/_3$; sev.; —; 3 mos. $^2/_3$; sev.; 430; 6 wks.	2 wks.: no varices; 1 mo. $^1/_3$; mild; 220; 7 wks. $^1/_4$; mild; —; 1 mo. no varices
4	Portacav.	$^2/_3$; sev.; 390; 10 days $^2/_3$; sev.; —; 3 wks.	2 wks.: no varices; 2 mos. no varices; 6 wks. no varices

510 to 300 mm. water in one case and from 530 to 254 mm. in the other (Table 6). The varices eventually disappeared from both.

COMMENT

The danger of encouraging dissemination of eggs through the body by creating a portacaval shunt has caused only minor concern. The presence of esophageal varices is good evidence that a high degree of portal venous collateralization has already occurred and that the liver filter is already being by-passed by eggs carried in the portal current. Portacaval anastomosis renders systemic egg dissemination more direct but, in the patient with varices, is not thought to alter the dissemination phenomenon appreciably.

A more difficult clinical problem revolves about the decision regarding proper chronology of treatment of active schistosomiasis and treatment of coexistent varices. Certainly, it appears best to treat the former as thoroughly as practicable before a vascular shunt is made. The danger of exsanguination from varices is such an imminent one, however, that the advantage of delay for schistosomiasis treatment may be considered only a relative one.

The important hiatus in understanding of esophageal varices is their natural history under conditions of static portal hypertension. There is nothing other than mere assumption to indicate that the size and distribution of varices remain unchanged if the portal pressure does not fluctuate. It was no surprise to find that successful schistosomiasis treatment did not affect the varices, particularly because rather good evidence has been presented to suggest that it is hepatic lobular regeneration rather than fibrosis which is responsible for venous obstruction in the cirrhotic liver (Kelty, Baggenstoss and Butt, 1950). Parenthetically, one notes that there is information to indicate that Fuadin does not increase the hepatic damage of schistosomiasis (Hernández-Morales, 1945).

Portal decompression by portacaval shunt has proved to be a feasible procedure for routine attack on esophageal varices and is considered to be indicated in selected cases as soon as varices are discovered, whether or not there has been a history of hemorrhage (Jahnke *et al.*, in press). The results have been most encouraging. Because in schistosomiasis both intra- and extrahepatic blocking mechanisms may be at work, however, the problem of planning proper surgical approach may be difficult. It has been found that routine percutaneous lienoportal venography is useful prior to operation, in order that fruitless exploration of a blocked portal vein may be avoided in favor of the next best procedure, splenorenal shunt.

Knowledge of the presence of esophageal varices is considered to be a vital matter. Radiologic methods have proved so impotent for their demonstration (Brick and Palmer, in press) that esophagoscopy has become a necessary part of the routine study of patients with those diseases which are sometimes accompanied by portal hypertension.

SUMMARY AND CONCLUSIONS

Forty-six active-duty soldiers, who were only recently well, were studied because of various abdominal symptoms and were found to have schistosomiasis

mansoni or *japonica* in differing stages of chronicity. Splenomegaly was the only clinical sign pointing to portal hypertension, but endoscopic investigation revealed esophageal varices in seven and gastric varices in one. Treatment of schistosomiasis did not effect any important general change in the appearance of the varices or in the portal venous pressure. Two patients were treated by end-to-side portacaval shunt, with disappearance of the varices.

It is concluded that schistosomal infection which is clinically mild may be accompanied by occult but important portal hypertension with esophageal varices. The danger of hemorrhage from unsuspected varices renders routine esophagoscopic study desirable in this infection. Portacaval or splenorenal shunt operations, as lienoportal venography may dictate, promise to be the most effective measures for treatment of varices secondary to schistosomiasis.

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MURRAY VALLEY ENCEPHALITIS: A SURVEY OF SUSPECTED MOSQUITO VECTORS[1]

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Encephalitis became epidemic in the Murray Valley of Australia early in 1951. Recognition of the disease, known as Murray Valley encephalitis (hereafter designated as M.V.E.), and isolation of the responsible virus stimulated extensive interest in the epidemiology. Studies were undertaken by Anderson (1952a, b) and his co-workers (Anderson *et al.*, 1952; French, 1952) and others (Miles *et al.*, 1951), and these led to the recognition of the epidemiologic pattern (Anderson, 1952b; Anderson and Eagle, 1953; Miles and Howe, 1953; Burnet, 1952). By the time the disease was identified, it was too late to investigate extensively one important possibility—that the virus might be carried by mosquitoes of the area. Some 550 mosquitoes of 4 species were collected by Mr. D. J. Lee and Dr. W. J. Stevenson in the Shepparton area of Victoria and examined for M.V.E. virus both in chick embryos and mice by one of us (E.L.F.) without isolating any virus. These collections were made in April, 1951, at the end of the epidemic period and were not continued when it was realized that the epidemic had subsided and further collections were unlikely to be rewarding.

Because of the behavior of arthropod-borne viral encephalitides in other areas—persistence from season to season, at least at an endemic level, usually in animal hosts or mosquitoes, and recurrence—it was important to pursue the entomologic aspects of M.V.E. Mosquitoes of the area were studied with their vector potentials in mind. The information gained has value in its negative significance and in its extension of knowledge of biology of the mosquitoes of this area. Description of the entomologic observations, virus tests and methods may assist further investigators of virus encephalitis, particularly M.V.E.

THE STUDY AREA

This was a limited region of northwest Victoria and southern New South Wales where clinical M.V.E. had occurred and where there had been serologic evidence of extensive inapparent infection in human and animal residents the previous year. The approximate center was the town of Mildura, and the area extended west to Wentworth, New South Wales, and south to Red Cliffs, Victoria. This encompassed an area along the Murray River (Figure 1) approximately 30 miles long extending back several miles from the river on the Victorian side and

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FIGURE 1. The Murray River in the area of study

FIGURE 2.* Residual flood waters in the natural flood plains river bottom of the Murray River.

* Reprinted by courtesy of the Australian Women's Weekly.

several miles up the Darling River on the New South Wales side. The habitats were widely varied—natural flood plains river bottom (Figure 2), intensively irrigated farmland, domestic environs, and towns.

The irrigation system provided numerous semipermanent or casual bodies of water ideally suited to mosquito breeding. In one area water was pumped from the Murray River into King's Billabong (an artificial lake in an old river channel), and from the billabong to the irrigation channels. In other areas water was pumped directly from the river to irrigation systems. The Mildura agricultural district was irrigated by water flowing through large open concrete channels and then through smaller channels with earthen banks. Water was liberated from the channels on each farm every few weeks and allowed to flow between the vines or fruit trees, or to flood low-lying pastures. Adjacent natural depressions and borrowpits formed in the construction of roads or channels would then fill with water, either by direct overflow or frequently by seepage through the channel banks. Every time the level of a river lock was raised to keep up the supply of water for irrigation, the river overflowed its low banks for some distance upstream, refilling adjacent waterholes and providing waterlogged ground where every hoofprint became a potential breeding place. Changes in the water level of King's Billabong also supplied water for mosquito breeding around the margins. Water draining from fruit blocks onto low-lying swampy ground or into lakes was of high salt content, and, with evaporation, some water had become brackish.

Tanks, wells and dams provided a much smaller source of man-made mosquito breeding places than those arising from irrigation or river flooding. Since it rained very little during the study period, the only available natural groundwater breeding places were large permanent swamps, billabongs and waterholes, and the river itself. Possibly some deep treeholes filled by earlier rains had not dried out when the study was begun, but all those examined were dry. One dry treehole was filled with water, and mosquito larvae were subsequently collected from it.

METHODS

Field collection of mosquitoes. Mosquitoes were collected in December, 1951, and January and February, 1952. Adults were collected from a great variety of natural and domestic environments. Daytime collections of resting females were of two types: (a) from shelters such as caves in earth banks, hollow trees and stumps, under bridges, in grass, and in areas away from domestic habitations; and (b) from man-made shelters such as fowl shelters, barns and other buildings. Mosquitoes were also caught at night, or occasionally in the day, by attracting them to human or animal bait. Most adult mosquitoes were collected with sucking tubes and transferred to bobbinette carrying cages for transporation to the laboratory. Larvae were collected by the usual methods.

Field preparation of mosquitoes for virus studies. Adult mosquitoes to be used in virus isolation studies were routinely held alive for 24 hours after collection to permit digestion of freshly taken blood meals. They were then anesthetized with chloroform vapor in sublethal doses. The species of each mosquito was identified

under the microscope, and each species was tubed separately in pools of 50 specimens or fewer, a convenient number for subsequent virus tests. Tubes were quickly sealed in a flame, labelled and stored in dry ice. Periodically, the tubes were packed in dry ice and shipped to the virus testing laboratory.

Virus isolation

Preparation of mosquitoes for inoculation. At the laboratory the mosquitoes were stored in dry ice until they could be tested. The tubes were then removed and opened at both ends, and the mosquitoes were forced out with a cotton wool plug. In the early phases of virus testing, each test pool contained a maximum of 50 mosquitoes, and in many pools an even smaller number. As studies progressed, the pool size was increased to 100 mosquitoes. They were ground in sterile chilled mortars with enough alundum to make a damp paste. To each pool of 50 mosquitoes, 2 ml. of calcium saline containing 5 per cent of inactivated rabbit serum was added (see below), and the mixture was reground for about 30 seconds. If the pools were larger, the volume of diluent was increased proportionally. The mixture was spun in an angle centrifuge at 2,500 r.p.m. for from 5 to 10 minutes and the supernatant fluid collected. One part of antibiotic solution was added to 4 parts of supernatant fluid, and this mixture was used to inoculate eggs and mice. The antibiotic solution contained 20 mg. of dihydrostreptomycin sulfate (Glaxo) per milliliter and 3,750 units of calcium penicillin (Commonwealth Serum Laboratories) per milliliter in physiological saline solution.

In preparing the 5 per cent rabbit-serum saline diluent, a solution composed of NaCl 9 gm., $CaCl_2$ anhydrous 0.25 gm., and distilled water to 1 liter, was sterilized by autoclaving. To this was added 5 per cent of normal rabbit serum, which previously had been heated to 56°C. for 30 minutes. The serum was tested on several occasions for neutralizing antibody to M.V.E. virus, with negative results.

Inoculation of chick embryos. The chorio-allantoic membrane of each of six 11-day-old chick embryos was inoculated with 0.1 ml. of each mosquito suspension according to the method of Beveridge and Burnet (1946). After incubation of the embryos at 35°C. for three days, the chorio-allantoic membranes were removed and examined for lesions. The membranes were then ground with alundum, suspended in rabbit serum saline, inoculated into 11-day-old chick embryos as before, and these embryos were incubated in the same manner. Again, the membranes were removed and inspected for the lesions produced by M.V.E. virus (French, 1952). If the tissues appeared normal after this second passage, they were discarded and the result was regarded as negative. If any suspicious lesions were seen, further passages were carried out.

Inoculation of mice. Six suckling mice 4 to 8 days old were each inoculated intraperitoneally with 0.1 ml. of mosquito suspension. The mice were observed for 21 days, and if evidence of infection was not found, the result was considered negative. Blind passages were not undertaken.

Prevention of cross infection. All attempts to isolate virus from mosquitoes were

TABLE 1

Species and number of female mosquitoes tested for virus
(December 2, 1951 to February 20, 1952)

SPECIES	DATE COLLECTED AND NUMBER TESTED						TOTAL	
	December		January		February			
	Pools	Specimens	Pools	Specimens	Pools	Specimens	Pools	Specimens
C. annulirostris	35	1,056	86	3,959	68	3,144	189	8,159
A. annulipes	44	2,088	37	1,599	34	1,561	115	5,248
*C. fatigans**	11	462	21	853	22	693	54	2,008
M. linealis	6	270	17	781	5	227	28	1,278
A. vittiger	—	—	—	—	1	25	22	564
A. theobaldi	3	8	10	352	3	49	16	409
A. camptorhynchus	1	50	2	11	1	14	4	75
A. alternans	—	—	2	27	—	—	2	27
M. uniformis	—	—	2	21	—	—	2	21
T. atripes	—	—	—	—	1	44	1	44
Total	100	3,934	198	8,142	135	5,757	433	17,833

* See text for discussion of this complex.

carried out in a separate room away from other work on M.V.E. virus. Hands were carefully washed with soap and water after handling each pool of mosquitoes

RESULTS

Virus isolations. Two hundred and forty-six pools (9,942 mosquitoes of 8 different species) of 50 or less were examined by inoculation of chick embryos. M.V.E. virus was not isolated. Since this procedure required a considerable amount of effort, time, personnel and material, the maximum number of mosquitoes in each pool was increased to 100. Then, an additional 187 pools, (7,891 mosquitoes of 10 species) were inoculated into chick embryos, bringing the total to 433 pools, (17, 833 mosquitoes of 10 species), and again M.V.E. virus was not isolated. The details are given in Table 1.

Seventy-four of the above 433 pools (3,165 mosquitoes of 8 species) were examined concurrently by inoculation into suckling mice, also with negative results.

Seven viruses producing pocks on the chorio-allantoic membrane and having the general characteristics of bird pox viruses were isolated (French and Reeves, 1953) from pools of "*Culex fatigans*,"[5] and one from a pool of *Culex annulirostris*. None of these viruses produced symptoms in infant or weaned mice.

When the decision was reached not to test all the mosquitoes collected and

[5] "*Culex fatigans*" refers to adults of the *Culex pipiens* group (Dobrotworsky, 1953; Dobrotworsky and Drummond, 1953). Owing to the close similarity of females of this complex, no attempt was made to separate the species before tubing. The two abundant members of the complex were *C. fatigans* and *C. pipiens australicus*.

prepared for virus isolation studies, the remaining 23,677 specimens in groups of 25 or more insects, representing 9 species, and aliquots of the material from pools of mosquitoes negative in M.V.E. tests were given to Professor F. Fenner of the National University of Australia to test for myxomatosis virus. He reports three isolations of this virus from 25 pools including a total of 12,568 *Anopheles annulipes* (Fenner, 1953). Serum was collected 21 days after inoculation from nine rabbits inoculated for myxomatosis virus with pools of *C. annulirostris* (of which a total of 9,515 were tested) and examined by one of us, both by complement fixation and neutralization tests, for antibody to M.V.E. virus. No antibodies were detected, supporting the conclusion that the mosquitoes contained no M.V.E. virus.

Biological observations

Adult mosquitoes. In collecting large samples of mosquitoes for virus studies, considerable data were obtained on the biology of adult mosquitoes of this region (Table 2). Since a wide variety of collecting technics was used and many different habitats were investigated, it was possible to collect 16 species. The only collecting technic with which all species were found was that of collection from cows, both in the evening and in the daytime. Other collecting methods were more selective and generally provided fewer mosquitoes.

Other species undoubtedly may occur in the area but the present study is believed to offer a detailed picture of the adult mosquito population during the study period. The accuracy and completeness were enhanced by the variety of collecting technics and of habitats sampled. Data on the distribution of different species attacking man, cow, and horse, and on the species in different environments are of particular value to epidemiologic studies of M.V.E. Some species commonly feed on all three of these hosts. The more common species, *A. annulipes*, *C. annulirostris*, *"C. fatigans,"* *Mansonia linealis* and *Aedes theobaldi*, frequently invade all habitats and feed on man if he is present.

Larvae. Studying the aquatic sources of mosquitoes provided information on the origin of the large populations of adult mosquitoes and preliminary information that could be used in the development of a mosquito control program. The following notes and Table 3 summarize the results of 71 collections of larvae, and the species collected as larvae, from 62 sites in the Mildura district. These cover all the different types of breeding places to be found in the district at that time of the year.

Larvae of *C. annulirostris* were found in grassy and weedy pools and hoofprints along the margins of the river and of permanent and semipermanent waterholes, but not among bullrushes (the main body of water in such places is usually well-stocked with fish and other predators); along the grassy or weedy margins of earthen drainage channels and in adjacent seepage pools; in flooded pastures and in shallow reedy or grassy swamps formed by irrigation drainage; in slightly brackish weedy pools of a salted drainage area; sometimes in casual temporary pools formed in natural depressions or borrowpits by overflow of irrigation water.

TABLE 2

Species and number of female mosquitoes collected from various habitats and baits

(November 30, 1951 through February 21, 1952)

TYPE OF COLLECTION	FEMALES COLLECTED																NUMBER OF COLLECTIONS
	Anopheles annulipes	*Culex annulirostris*	*Culex fatigans**	*Culex cylindricus*	*Mansonia linealis*	*Mansonia uniformis*	*Aedes theobaldi*†	*Aedes camptorhynchus*	*Aedes villiger*	*Aedes alternans*	*Aedes sagax*	*Aedes vigilax*	*Aedes notoscriptus*	*Aedes tremula*	*Aedes bancroftianus*	*Trip. atripes*	
Natural shelters	1,830	881	545	6	96	4	37	6	405	86	12	—	1	—	—	—	47
General domestic shelters	2,779	1,052	3,211	—	30	—	12	21	—	13	—	2	—	1	—	2	100
Chicken houses	1,974	923	2,136	—	46	—	9	22	2	—	—	—	—	—	—	—	65
Cow bait	6,378	15,013	11	20	4,019	183	1,145	205	724	80	2	34	1	1	1	1	102
Human bait	541	762	29	—	42	—	18	—	25	4	1	2	8	3	—	104	25
Horse bait	158	165	—	—	19	—	40	9	—	1	—	—	—	—	—	—	4
Miscellaneous collections	472	279	122	—	24	2	19	323	9	—	—	1	—	—	—	—	8
Total	14,132	19,075	6,054	26	4,276	189	1,280	586	1,165	184	15	39	10	5	1	107	351

* See text for discussion of this complex.

† A single female specimen of *Aedes theobaldi eidsvoldensis* was collected.

TABLE 3

Larval habitats of mosquitoes in the Mildura district
(December, 1951 through February, 1952)

	LARGE PERMANENT SWAMPS, BILLABONGS OR WATERHOLES	SEMIPERMANENT SWAMPY AREAS OR WATERHOLES	CONCRETE DRAINAGE CHANNELS	EARTHEN DRAINAGE CHANNELS AND DITCHES	FLOODED PASTURES AND DRAINAGE SWAMPS	SEEPAGE POOLS FROM DITCHES OR SWAMPS	CASUAL POOLS	BRACKISH POOLS	RIVER MARGIN POOLS AND HOOF PRINTS	SEEPAGE POOLS FROM HOUSEHOLD DRAINAGE	UNDERGROUND TANKS AND WELLS	RAINWATER TANKS AND ARTIFICIAL CONTAINERS	TREEHOLES	TOTAL
Total number of sites...	8	7	2	7	10	4	6	3	2	1	3	8	1	62
A. annulipes.....	8	7	2	6	9	4	2	1	2	—	1	1	—	43
C. annulirostris.....	4	5	—	4	9	3	3	1	2	—	—	—	—	31
C. pipiens australicus.....	—	1	1	3	7	3	5	—	1	—	1	1	—	23
*C. fatigans** (typical).....	—	—	—	—	—	—	—	—	—	1	3	7	—	11
*C. globocozitus**.....	1	—	—	—	—	—	—	1	—	—	—	—	—	2
A. alternans.....	—	2	—	1	3	1	3	—	—	—	—	—	—	10
A. vittiger.....	—	—	—	1	2	1	1	—	—	—	—	—	—	5
A. theobaldi.....	—	1	—	2	3	—	2	—	—	—	—	—	—	8
A. camptorhynchus..	—	—	—	—	—	—	—	3	—	—	—	—	—	3
A. notoscriptus.....	—	—	—	—	—	—	—	—	—	—	—	—	1	1
A. tremula.....	—	—	—	—	—	—	—	—	—	—	—	—	1	1
T. atripes.....	—	—	—	—	—	—	—	—	—	—	—	—	1	1

* See text for discussion of this complex.

A. annulipes was found in all types of breeding places frequented by *C. annulirostris* and in some other sites. In permanent or semipermanent waterholes these larvae can sometimes find enough shelter from predators among bullrushes or surface vegetation in the more open water. They were also collected in concrete drainage channels along the margin of a flowing channel and in residual pools in an empty one, and in an underground iron tank and a rainwater drum.

Three forms of the *C. fatigans* complex were found: (1) Typical *C. fatigans* larvae were found in domestic situations, often in polluted water, in underground tanks and wells, rainwater tanks and artificial containers, and in seepage pools formed by household waste water. (2) *C. pipiens australicus* larvae were found in all types of ground water except permanent waterholes, usually associated with *C. annulirostris* and *A. annulipes*, but were not found as frequently as these two species except in casual pools where they were commoner. They were also found in an underground tank, in a fowlyard water trough and in residual pools in a concrete channel. (3) *Culex globocozitus* larvae were found in slightly brackish weedy pools on a salted drainage area and among bullrushes on the margin of a permanent swamp. This form may be more widespread than the present records indicate because the larvae are very similar to *C. pipiens australicus* and might be overlooked when associated with that species.

Certain *Aedes* species breed, under normal circumstances, in casual groundwater standing after heavy rain or river floods. Usually, one generation breeds through and another does not appear until the breeding place has dried out and refilled. In the Mildura agricultural district, as already described, many temporary pools are formed by waste irrigation water, so the species no longer depend on rainfall for breeding places and hence can breed continuously in the newly formed casual pools. The species in such sites were *Aedes alternans*, *Aedes vittiger* and *A. theobaldi*. *A. vittiger* breeds through more rapidly than the other two species. Similarly formed pools on salt flats, where the water is brackish, are sources of *Aedes camptorhynchus*, found also on one occasion in a clay-pit in saline pools formed by overflow waters from a brickworks. *Aedes notoscriptus*, *Aedes tremula* and *Tripteroides atripes* were found in a rot hole in the stump of a eucalyptus. Treeholes are the normal breeding places of these species, but all three may breed in artificial containers.

Larvae of seven species could not be located, but their source can be conjectured on the basis of studies in other areas of Australia. All known *Mansonia* larvae and pupae, for example, attach themselves to the roots or stems of aquatic plants, from which they obtain their oxygen. The larvae of *Mansonia uniformis* have been collected from a variety of plants. The larvae of *M. linealis* have not yet been discovered. There is little doubt that larvae of both species are associated with aquatic plants in King's Billabong and other swamps near which numerous adults were collected, but repeated search failed to discover their immature stages. *Aedes sagax* larvae have been found in casual groundwater. *Aedes vigilax* commonly breeds on the coast in brackish pools on tidal flats, but occasionally breeds in casual fresh-water pools. It almost certainly breeds in the Mildura district on flooded saltpans, since numerous adults were taken in that vicinity. The adults were found in the same area as *A. camptorhynchus*. *Aedes bancroftianus* breeds in casual ground pools. Only the female of *Aedes theobaldi eidsvoldensis* is known. It almost certainly breeds in casual fresh-water pools. *Culex cylindricus* breeds elsewhere in shaded pools in fresh-water streams.

DISCUSSION

The Mildura area was selected for intensive entomologic investigations because it was a most likely locality for the continued activity of M.V.E. virus in mosquitoes, if they serve as vectors. At the end of the epidemic the previous summer, 20 per cent of 330 normal adult residents gave serologic evidence of this virus infection. Many animals of the area had antibodies (Anderson *et al.*, 1952). The possibility was promising that this might remain an endemic focus where natural virus transmission could be studied.

The failure to isolate virus from a large sample of mosquitoes collected in the season of expected virus activity may be attributed to one of two factors: either the mosquitoes examined did not carry the virus, or the isolation techniques were not adequate. Use of the developing chick embryo is a sensitive method of isolating M.V.E. virus. Three strains had been isolated from human brain tissue by the procedure used (French, 1952). In two of these isolations virus was not detected in simultaneous tests with mice. Furthermore, isolation

on the chorio-allantoic membrane of seven strains of a different virus from the mosquitoes indicates that the technique is adequate for isolation of at least some viruses normally carried by mosquitoes of that area. In experiments on the ability of various mosquitoes to become infected with M.V.E. virus (McLean and French, 1953), isolations were made from *A. notoscriptus* infected and incubated for seven days in the laboratory and subsequently handled like the field-collected mosquitoes in the present study, including ten days storage on dry ice.

The role of mosquitoes as possible vectors of M.V.E. virus is still to be determined. All epidemiologic information indicates that it could be mosquito-borne. The following characteristics of the infection are particularly relevant: its seasonal and geographic distribution, the distribution of clinical and inapparent infection in man and a variety of animal hosts, and its close immunogenic relationship to a virus causing a known mosquito-borne disease, Japanese B encephalitis. As might be anticipated, field investigations at the conclusion of and following the epidemic did not furnish the necessary proof, nor provide essential data on the prevalence of possible vector populations preceding or during the onset of the epidemic. In the absence of such information, the alternative is to interpret data of the type collected in the present study.

The activities of at least several species of mosquitoes could explain the epidemiologic pattern of spread during the epidemic. During the summer several species were found in large populations; they occurred in the complete range of habitats investigated; and there was ample evidence of their willingness to feed on a diversity of hosts, including man and possible animal "reservoirs" of the virus.

The following observations on several species lead to their incrimination as possible vectors. Particularly striking is the gross morphologic and biologic resemblance of *C. annulirostris* to species such as *Culex tarsalis* and *Culex tritaeniorhynchus*, primary vectors of encephalitis viruses in other areas of the world. *Culex annulirostris marianae*, a subspecies in the Pacific area, is incriminated on epidemiologic evidence as a possible vector of Japanese B virus on Guam (Reeves and Rudnick, 1951). *C. annulirostris* from the Caroline Islands is an experimental vector of this virus in the laboratory (Hurlbut and Thomas, 1949). *C. annulirostris* is one of the three most common species of mosquito in the Murray River Valley during the summer. Adults and larvae are common in a wide variety of habitats, ranging from river bottom to domestic surroundings. The species commonly feeds on man as well as a wide range of other mammalian and avian hosts.

In the present survey *A. annulipes* and the *C. fatigans* complex were the other two predominant members of the mosquito population in the study area. If one considers, for epidemiologic purposes, that the *C. fatigans* complex is a single species (and this may not be warranted), it had the same biologic attributes as *C. annulirostris*. In other areas the domestic representative of the complex, *C. fatigans* (=*quinquefasciatus* in the Americas), is an experimental vector of St. Louis (Smith *et al.*, 1948; Reeves, 1953) and Japanese B viruses (Reeves and Hammon, 1946; Hurlbut and Thomas, 1949), and has been found naturally infected with the St. Louis virus (Reeves, 1953). The relationship of *A. annulipes*

to M.V.E. virus has recently been studied and this mosquito has been found to be unable to carry the virus under laboratory conditions (McLean, 1953).

Species of *Mansonia* and *Aedes* cannot be excluded from future considerations. *M. linealis* was sufficiently prevalent in many environments to warrant its investigation. In the Americas, *Mansonia titillans* is a primary vector of Venezuelan equine virus (Gilyard, 1944) and *Mansonia perturbans* has been found naturally infected with Eastern equine encephalomyelitis virus (Howitt *et al.*, 1949). Several species of *Aedes*, but especially *A. theobaldi*, fit the epidemiologic pattern, but the population was too small to warrant serious consideration. It is more difficult to relate current populations of *Aedes* to those that may have existed during the epidemic than it is to do so for species of *Culex*, *Anopheles* or *Mansonia*.

Aedes populations depend so completely on temporary water sources, such as river flooding, heavy rainfall and pasture flooding by irrigation, that their populations are not stable from year to year. The other genera, more commonly originating from permanent or semipermanent waters, are most likely to be in constant numbers and comparable from year to year because their populations remain relatively high, even in unfavorable years. The present study does not lead to the belief that *Aedes* were important. The aquatic conditions and populations may have been quite different during the epidemic.

The weather in the Mildura area was not unusual immediately before or during the epidemic, nor in the time of the present study. Average summer temperatures were uniformly sufficiently high to permit extrinsic incubation of a virus in a vector. Summer rainfall in both the epidemic and following years in this limited area was insufficient to affect mosquito populations significantly. One factor that did differ in the two years was the flood stages of the river, a reflection of rainfall in the distant headwaters. In the epidemic year this was abnormally high; in the following year it was below normal. This undoubtedly would be reflected in the mosquito populations of the river bottom land and its margins—extensive sources of mosquito larvae when inundated by floods. However, it should be emphasized that in a region of this type, where irrigation is carried out on a large scale, the volume of water on the extensive agricultural land area varied little during the two years.

Of the mosquitoes in the Mildura district, those that can adapt to the most varied breeding places are undoubtedly *C. annulirostris* and *A. annulipes*. This is reflected in the numbers of adults collected. Both species were collected from all types of groundwater breeding places produced by irrigation. Although *A. annulipes* was more frequently found in these collections, where both occurred *C. annulirostris* larvae were usually more numerous. In some places tremendous numbers of the latter species were breeding. Permanent control measures against one or both of these mosquitoes would require modification of the present irrigation system.

Populations of supected mosquito vectors equalled or exceeded those observed in areas of the United States or Pacific Islands by the senior author where other arthropod-borne viral encephalitides are endemic.

Studies by Anderson and Eagle (1953) concurrent with and following these entomologic studies offered no evidence of virus activity in the vertebrate hosts commonly infected during the epidemic. All of these studies lead to the conclusion that the virus had vanished from the Mildura region in the year following the epidemic. Hypotheses concerning the possible endemic sites of this virus and the methods by which it could be maintained and spread have been developed independently and concurrently by Anderson (1952a, b), Anderson and Eagle (1953) and Miles (1953) and discussed by Burnet (1952).

Proof of the possible role of mosquitoes will depend on their study in areas where the virus is endemic or epidemic and on careful laboratory evaluation of their vector ability.

SUMMARY

A survey of the mosquitoes of a section of the Murray River Valley of Australia has provided data of value to epidemiologic investigators of Murray Valley encephalitis and to persons possibly concerned with mosquito problems in this area.

In these studies, 16 species of mosquitoes are recorded. Laboratory tests for Murray Valley encephalitis virus on 17,833 mosquitoes representing 10 species have been negative. Several other types of virus, an unclassified pox virus and myxomatosis virus, were isolated from mosquitoes collected.

Interpretation of data on the biology of adult mosquitoes permits some evaluation of the relative potential importance of the various species as vectors of Murray Valley encephalitis. It is concluded that certain species should be suspected as vectors, in the following order of importance: *Culex annulirostris*, *Culex fatigans* "complex," *Mansonia linealis*, *Aedes theobaldi* and possibly *Anopheles annulipes*. As no epidemiologic evidence could be found that the virus was still present in the area studied, the negative findings neither prove nor disprove that mosquitoes are vectors of this virus, and further studies are warranted.

A sample of 71 larval collections has been analyzed as to larval habitats, association of various species, the conditions of water source and use leading to mosquito breeding and possible control problems. It is suggested that an effective program for mosquito control would require modification of existing irrigation facilities and practices, as well as an attack on natural and purely domestic water sources of mosquitoes.

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NOTES ON THE BITING RESPONSE OF TSETSE FLIES

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Since one of the limitations on the capacity of the tsetse fly to serve as an effective vector of human and animal trypanosomiasis is the number and variety of chosen hosts, the physiology of host selection is a matter of some immediate importance. If one may be permitted an analogy with parasitoid and plant-feeding insects, it becomes apparent that host selection is a complex behavior pattern involving the interaction of many stimulus/response components. Host selection reduced to its simplest common denominator involves long and short range orientation followed by a stimulus to feed. Factors which lead an insect to the vicinity of its host may differ widely from those operating in the final phases of orientation (*cf.* von Frisch, 1923, 1950, 1951; Fraenkel and Gunn, 1940; Salt, 1935; Ullyett, 1936; and Dethier, 1953). Upon successful completion of orientation, still other factors initiate biting response and continued feeding. The behavior of the tsetse is no less complex.

The factors which operate in long range orientation have received some attention. Within a given area a fly seeks out from among available hosts one or more species for preferential feeding (Vanderplank, 1944; Jackson, 1949). Vision appears to be of prime importance in the location of animals (Nash, 1930; Bax, 1937), but the olfactory sense also plays a part, though perhaps a subsidiary one (loc. cit., also Chorley, 1948). However, the stimuli which initiate the act of biting once a host has been located have not been investigated. The study reported here is concerned with this component of the feeding behavior.

MATERIAL AND METHODS

Three species of tsetse flies were studied: *Glossina palpalis* Robineau-Desvoidy, *G. morsitans* Westwood, and *G. brevipalpis* Newstead. These are representative members of the three groups into which the genus is usually divided, namely: the *palpalis*, *morsitans*, and *fusca* groups respectively. Specimens of *G. palpalis* were captured each morning in banana plantations along the banks of the rivers Grande Ruzizi and Petite Ruzizi and in the gallery forests along the river Mpanda. Specimens were also taken on the rivers Kalimabenge, Kawezi and Kiliba. All of these streams debouch into Lake Tanganyika in the vicinity of Uvira, Belgian Congo. *G. morsitans* and *G. brevipalpis* were captured in the Ruanda Urundi near the Tanganyika border in the region of the Malagarasi River.

Flies received into the laboratory were fed on guinea pigs until ready for use. Thus, while the age of flies in any given experiment varied, the nutritional state was controlled. Unless stated otherwise below, all flies were tested 24 hours after having engorged.

All experiments were conducted either with flies caged in Bruce boxes or with

flies attached individually to sticks. These last were mounted according to a technique first applied to muscoid flies by Minnich (1926) (*cf.* also Dethier and Chadwick, 1947). For the present study flies were lightly etherized and attached by their wings to wax-tipped applicator sticks. Flies fixed in this manner fed normally. If unfed, they frequently survived longer than caged flies probably because of enforced inactivity. Females produced viable puparia. If freed from the sticks, both sexes copulated normally. As this study will show, there is an enormous advantage in being able to work with fixed flies. The technique permits of easy handling of infected flies, of close observation of behavior and of following individuals through a variety of tests. Parallel experiments in the form of tests with caged flies serve as control against any idiosyncrasies of behavior attendant upon the restriction of movement. Despite the obvious limitations of the technique it is very useful in physiological studies and its further application to the study of *Glossina* should be rewarding.

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NORMAL BITING RESPONSE

When a tsetse fly alights upon its host, it seldom moves from its landing site. Unlike some other bloodsucking Diptera (*e.g.* certain species of *Tabanus*) it makes no exploratory excursions. A rather complex behavior pattern is displayed preparatory to feeding. The first noticeable reaction is an increased vibration of the halteres. After a period of waiting, which varies from a fraction of a second to as much as thirty seconds and varies somewhat with the physiological state of the fly and the species, a small drop of salivary fluid is extruded from the tip of the mouthparts. The entire mouthparts are then depressed toward the substrate, the haustellum becomes disengaged from the palpi and piercing commences. Pumping of blood then begins and products of earlier digestion are extruded from the anus. One or more of these components of behavior may be omitted from the chain of events or the sequence may be interrupted at any point. For example, some flies salivate and do not probe. Many probe immediately without first salivating. Some depress the entire feeding apparatus before disengaging the haustellum or may fail to disengage it. Failure to free the haustellum is sometimes due to mechanical difficulties even in wild flies. Under the circumstances, feeding is impossible. Other flies disengage the haustellum as their first act and begin piercing immediately. Some defecate prior to probing, some during and some after. A few defecate and then do not feed. But whether the pattern is complete or not, it is constituted by the aforementioned com-

ponents, and these can be elicited by certain normal and artificial stimuli. Consequently, they serve as conspicuous signals marking the effectiveness or inadequacy of test stimuli. In the following experiments three reactions have been selected as criteria for the adequacy of a stimulus: salivation, depression of the entire mouthparts (hereafter referred to as probing), and disengaging of the haustellum (hereafter referred to as piercing).

EXTERNAL FACTORS INITIATING BITING RESPONSE

Substrate. Preliminary tests were undertaken to determine the role of contact alone in eliciting biting responses, since contact with a neutral surface was to be used in subsequent experiments as a control. Excessively smooth surfaces such as glass proved to be unsatisfactory. They inhibited responses probably because the fly was constantly striving to secure purchase for its feet. Conversely, a rough fibrous surface such as soft filter paper was equally unsatisfactory because the cellulose fibers entangled the fly's feet. Flies exhibited greatest tranquility when placed upon a surface which allowed them to secure a firm, unencumbered foothold. For experimental purposes a hard non-glossy paper such as bond was most satisfactory. This was therefore selected for control experiments. Moreover, since white was known from field tests to be least attractive to flies, it was chosen as the color to be used, with the idea in mind that any stimuli which would operate in the face of such color repellency could be considered stimuli of some weight. Beyond this, colors were not investigated.

Fixed flies were found to give a low order of response to paper substrates of this sort. Care was taken to prevent handling of the paper since the possible role of odor had not yet been established. Of 439 *G. palpalis* tested, 22.7% probed the paper, 4.5% pierced it, and 4.5% salivated. In the case of 141 *G. morsitans* tested, 3.5% probed, 2.1% pierced, and 1.4% salivated. With *G. brevipalpis* 14.4% probed, 0% pierced, and 0% salivated. It would appear, therefore, that with all three species contact of the tarsi with a chemically neutral substrate at ambient temperature exerts some power to evoke feeding responses. But this power is of a low order of magnitude, and, as will be seen later bears a relation to the nutritional state of the insect. Furthermore, it appears that some surfaces are definitely repellent in the sense that contact with them fails to evoke any components of the feeding response.

Contact chemicals. Tests were now undertaken to determine whether or not the three species of *Glossina* possessed tarsal chemoreceptors and, if so, whether these were involved in the feeding reaction. Again, fixed flies were employed. The results were uniformly negative. No reactions could be obtained using the following substances: water, sucrose, glucose, fructose, sodium chloride, urea, acetic acid, assorted amino acids and human sweat. The compounds were applied to bond paper which had previously been tested dry. In no case was the per cent response significantly different from that obtained in the control experiments. When the flies were tested by placing their feet on the surface of the respective solutions there were fewer responses than to the paper. This last result is further confirmation of the importance for biting reactions of contact with a

suitable substrate. When additional tests were arranged so that unattached caged flies could walk upon substrates impregnated with various compounds, the results were again uniformly negative.

Failure to obtain a response to such compounds as water and sugar is somewhat surprising in view of the fact that tarsal receptors sensitive to these compounds have been demonstrated in other bloodsucking Diptera. Frings and O'Neal (1946) demonstrated the sensitivity of the tarsi of *Tabanus sulcifrons* to carbohydrates and salts. Carbohydrates elicited extension of the proboscis; salts prevented extension. Krijgsman (1930), working with *Stomoxys calcitrans*, found evidence that there were tarsal receptors which responded to stimulation by serum and caused proboscis extension. He stated, however, that his results were not conclusive. Frings and Hamrum (1950) showed that both sexes of *Aedes aegypti* possessed tarsal receptors sensitive to stimulation by sugar. But, whereas males respond readily while attached to sticks, females fail to do so and their sensitivity could be demonstrated only by observing the reaction of free specimens walking on a substrate moistened with spots of water and spots of sugar.

In view of these last mentioned results, even though tsetses showed no reaction under similar circumstances, the failure to obtain a response should not be accepted as final even though under the conditions of testing the evidence is unequivocally negative. It would also be profitable to retest *Stomoxys*.

Odor. Since odors are known to elicit biting reactions in bloodsucking species (*cf.* Krijgsman, 1930), the biting reactions of tsetses to host odors were investigated. No reactions could be obtained to human and guinea pig odors when responses to a piece of clean muslin and a piece of muslin which had been worn as a coat for a week by a guinea pig were compared. There was no significant difference between the odorous and the odorless cloth. Nash (1930) had observed earlier the reluctance of *G. morsitans* to feed after its antennae had been covered with shellac and he attributed this reluctance to interference with olfactory receptors. Present evidence indicates that failure to feed results instead from extirpation of the thermal receptors.

Heat. The effectiveness of heat was tested with a tube through which was circulated water heated to a temperature of 45° C. The tube was tightly wrapped in one thickness of white bond paper. The same kind of paper wrapped around an empty tube at room temperature served as a control. Under these conditions of testing the temperature at the surface of the hot tube was approximately 38° C. Attached flies were tested by holding each in turn with its tarsi touching the warmed paper for one minute. Two controls were run: one, the empty paper-covered tube, and the other, the finger of the experimenter. Thus, biting responses were tested under three different conditions. Either probing or piercing was accepted as a criterion of response. Collected data for the three species are presented in Table 1.

It is clear that the heated tube and the finger were much more stimulating than the tube at room temperature. The number of flies which probed was invariably increased; the number which pierced was increased in the majority of

TABLE 1

Biting responses of three species of tsetse flies

SPECIES	CONDITIONS COMPARED	NO. OF FLIES TESTED	MEAN % RESPONDING	PROBABILITY	SIGNIFICANCE OF DIFFERENCE
G. palpalis					
	control	439	27.2	<0.01	+
	finger	439	44.6		
	control	439	27.2	<0.01	+
	heat	439	47.3		
	finger	439	44.6	>0.5	−
	heat	439	47.3		
	finger	643	30.1	<0.05	slight
	finger + repellent	643	25.0		
G. morsitans					
	control	141	5.6	<0.01	+
	finger	141	17.7		
	control	141	5.6	<0.01	+
	heat	141	29.0		
	finger	141	17.7	<0.05	slight
	heat	141	29.0		
G. brevipalpis					
	control	69	14.4	<0.01	+
	finger	69	37.9		
	control	69	14.4	<0.01	+
	heat	69	43.4		
	finger	69	37.9	>0.5	−
	heat	69	43.4		

cases. When the two classes of response are combined, there is no doubt that the finger and the tube were providing powerful stimuli. Similar results were obtained when a guinea pig was the source of the stimulus. The increase in responsiveness brought about by the guinea pig and finger and lacking when odorous cloth was the test substrate indicates the superiority of heat as a stimulus. Furthermore, it is noteworthy that the hot tube was somewhat superior to the other objects. This may have been due to the fact that the tube was actually hotter than either the finger or the guinea pig. It was not possible to execute an exact study of the optimum temperature range for the feeding reactions. However, preliminary experiments showed that with an ambient temperature of 26 degrees a tube surface temperature of 34 degrees was necessary before responsiveness increased appreciably. A tube surface temperature in excess of 45 degrees inhibited response. This would indicate that the optimum temperature

lay between these two extremes. The preferred temperature range was thus rather narrow but compares favorably with the preferred ranges recorded for other bloodsucking arthropods (*cf.* Totze, 1933; Nieschulz, 1934; Homp, 1938).

The incidence of salivation was significantly higher with the hot tube than with the finger or guinea pig. This rise may be explained by the fact that salivation is a response not alone to favorable stimuli but also to unfavorable stimuli. Flies responded to contact with temperatures as high as 70°C. by salivating. As will be shown later, they responded to chemical repellents in the same manner. It has been for this reason that most comparisons of the effectiveness of stimuli have been in terms of the combined probing and piercing response to the exclusion of salivation. In this connection it is interesting to recall that flies could be made to salivate on a hot microscope slide where the drop of fluid could then be examined for trypanosomes. This procedure offers one method of diagnosing certain stages of the infection of a fly without killing it and may be of use where the insect must be used for subsequent transmission experiments.

The foregoing experiments with fixed flies were confirmed with free flies in a Bruce cage. The results were in complete agreement. Caged flies aggregated to warm objects and attempted to pierce them. They showed no appreciable reaction to muslin cloth which had been worn by a guinea pig for a week. All three species of flies behaved similarly.

The reaction of *G. palpalis* to heat is especially interesting in view of the fact that it feeds to a large extent on such cold blooded animals as the crocodile (Jackson, 1949). It is also interesting in this connection to recall that some workers (*cf.* Nat'l. Res. Council Rpt. No. 7) succeeded in inducing *Aedes aegypti* to feed on the American toad (*B. terrestris*) by raising the temperature of the skin. Much more could be done with *Glossina* by way of investigating precisely the optimum temperature range and the relation between this and the skin temperature of the preferred hosts.

INTERNAL FACTORS INITIATING BITING RESPONSE

The inclination of the tsetse to bite is affected by the internal physiological state. The state of hunger and that of water loss are the more obvious variables which might be concerned. It is stated (Jack, 1939; Jackson, 1949) that hunger in the tsetse is really an expression of water loss. The tsetse takes in no water as such and restores its water balance by a blood meal. Consequently, two variables which are of importance in the present considerations are the time since engorgement and the ambient factors which affect water loss, namely, temperature and humidity. It was not possible to control temperature and humidity; however, the responsiveness of flies to food stimuli could be studied as a function of duration of blood deprivation. During the period of the experiment the ambient temperatures and humidities varied only slightly so could be counted as exerting the same order of influence as deprivation.

To ascertain the effect of hunger on the feeding response, flies were given a blood meal and then tested at intervals till death. Since time alone is not a reliable index of the state of hunger, use was also made of Jackson's (1933) index.

This index is based on the appearance of the abdomen. The general pattern of response was similar for all three species of flies. Immediately after engorgement (stage I) and up to approximately 24 hours the response was low. From approximately 24 to 90 hours (stages III to IV) the response was maximum. After 90 hours (stage V) the response dropped sharply. Examination of those specimens which died showed that the majority died in stages IV to V. Coincidentally, responses to control substrates increased markedly. In short, the biting response increased as the flies became hungrier until they reached the penultimate stages of starvation. Approaching this point they became refractory, and the period probably marked the beginning of the moribund state. The trend of responsiveness agrees well with field observations of the relation between hunger and biting.

LOCATION OF THERMAL RECEPTORS

The high degree of sensitivity to heat over a rather narrow temperature range raises questions concerning the nature of the stimulation and the location of the receptors. Attempts to locate the receptors involved were made by following changes in responsiveness to heat after amputation of various appendages. In different experiments antennae, palpi, and both together were removed. From an examination of Table 2 it may be seen that removal of both antennae reduced the per cent of biting response to the finger and to heat to a value hardly different from the response to the control tube. It also reduced the response to the control tube to a value slightly below that expected of normal flies (*cf.* Table 1). Since normally there is some response to contact alone at ambient temperatures

TABLE 2

Biting responses of G. palpalis *following removal of various appendages*

CONDITION OF EXPERIMENT	CONDITIONS COMPARED	NO. OF FLIES TESTED	MEAN % RESPONDING	PROBABILITY	SIGNIFICANCE OF DIFFERENCE
Antennae removed					
	control	397	17.6	>0.1	–
	finger	397	22.4		
	control	397	17.6	<0.05	slight
	heat	397	35.5		
Palpi removed					
	control	146	36.3	<0.01	+
	finger	146	51.3		
	control	146	36.3	<0.01	+
	heat	146	51.3		
Palpi and antennae removed					
	control	265	23.7	>0.1	–
	finger	265	33.6		
	control	265	23.7	<0.02	slight
	heat	265	33.9		

it is not surprising that a residual response remained after removal of the antennae. The fact that even this was reduced somewhat indicates that removal of the antennae *per se*, quite aside from their possible role as bearers of thermoreceptors, has an adverse effect. However, the very great reduction to the finger and to heat following antennectomy suggests that the antennae do indeed function in thermoreception. That the operation itself was not the main contributing cause was shown by an experiment in which free flies were tested first following unilateral antennectomy and then bilateral antennectomy. In the first instance there was no reduction in response. Of 38 flies 80 per cent pierced and fed on a guinea pig. The following day the remaining antenna was removed and the response dropped to zero and remained so till all flies had died. The results would indicate, therefore, since removal of both antennae reduced the response to a finger to that normally elicited by the control tube, that the chief site of thermal receptors mediating the biting response had been abolished. The fact that antennaless flies showed to a heated tube a response which was somewhat greater than that elicited by the control suggests that other receptors remained. To test this possibility, flies lacking palpi and those lacking both palpi and antennae were tested. Data in Table 3 show that removal of the palpi did not reduce the response to control substrate, finger, or heat. As a matter of fact, there was a slight tendency for the response to be increased. Removal of palpi and antennae together reduced response to the finger and to heat. This reduction was greater than that observed with palpectomy alone but not significantly different from that observed with antennectomy alone.

The increased response following palpectomy requires explanation. In general fixed flies responded at a lower rate than free flies. It appears that this was due, at least in part, to mechanical difficulty. On the whole, fixed flies salivated and regurgitated more frequently than free flies, and their mouthparts tended to become stuck and occluded more often. Observation of individual flies showed that many which attempted to pierce were prevented from doing so by their inability to free the haustellum from the palpi despite strenuous efforts. If they were assisted, piercing proceeded normally. Removal of the palpi by amputation removed part of the source of this mechanical difficulty. Consequently, it is only natural that flies which had undergone palpectomy would have exhibited a higher per cent of response than the normal controls. The per cent of response of palpectomized flies was nearly identical to that of free flies in a cage.

The results indicate that the palpi contribute little to the receptor supply necessary for complete feeding reaction. The antennae are the principal site of these. There seem to be no thermoreceptors in the tarsi which function in this connection, although the feet are assuredly possessors of some thermoreceptors. At least they are sensitive to heat as evidenced by the fact that the feet are withdrawn from a heated surface. The action may not be mediated through specific receptors but rather through sense organs analogous to pain endings (*cf.* also Slifer, 1951, with regard to sensitive areas in the locust).

Confirmation of the experiments with fixed flies was obtained from comparable experiments with free flies. Those lacking palpi were attracted to and feed

TABLE 3

Biting responses of G. palpalis *to various stimuli following removal of different appendages*

NATURE OF STIMULUS	CONDITIONS COMPARED	NO. OF FLIES TESTED	MEAN % RESPONDING	PROBABILITY	SIGNIFICANCE OF DIFFERENCE
Control substrate					
	control	439	27.2	<0.01	+
	minus ant.	397	17.6		
	control	439	27.2	<0.01	+
	minus palpi	146	36.3		
	control	439	27.2	>0.5	−
	minus ant. and palpi	265	23.7		
	minus palpi	146	36.3	<0.01	+
	minus ant. and palpi	265	23.7		
Finger					
	control	439	44.6	<0.01	+
	minus ant.	397	22.4		
	control	439	44.6	>0.1	−
	minus palpi	146	51.3		
	control	439	44.6	<0.01	+
	minus ant. and palpi	265	33.6		
	minus palpi	146	51.3	<0.01	+
	minus ant. and palpi	265	33.6		
Heat					
	control	439	47.3	<0.01	+
	minus ant.	397	35.5		
	control	439	47.3	>0.5	−
	minus palpi	146	51.3		
	control	439	47.3	<0.01	+
	minus ant. and palpi	265	33.9		
	minus palpi	146	51.3	<0.01	+
	minus ant. and palpi	265	33.9		

normally to completion on a guinea pig. Those deprived of their antennae or their antennae and palpi never fed even though they frequently by chance came to rest upon the guinea pig. They eventually starved.

It is interesting to compare the responses of *Glossina* with those of mosquitoes.

Many workers (*e.g.* Howlett, 1910; Marchand, 1918; Crumb, 1922; Thomson, 1938; Peterson and Brown, 1951; Roth, 1951) have shown that various species of mosquitoes are attracted to and incited to probe by heat. Roth has shown that females of *Aedes aegypti* are no longer attracted to heat from a distance if antennectomized but will continue to probe if either the antennae or palpi are intact. That is to say, antennectomy and palpectomy in almost all cases completely abolish the probing response. Females of *Anopheles quadrimaculatus*, on the other hand, if antennectomized cannot locate their host, but will continue to probe a warm object even after complete antennectomy and palpectomy. *Glossina* is obviously more dependent on its antennae for initiation of the probing response than either of the mosquitoes.

THE ACTION OF REPELLENTS

The repellent 622 (6 parts dimethyl phthalate, 2 parts indalone, 2 parts 2 ethyl hexanediol-1,3) when applied to a shaven guinea pig prevented tsetse flies from feeding on the animal for at least two hours. Tests were conducted therefore with fixed flies for the purpose of learning something about the method of action of this material. A comparison was made of the reactions of fixed flies to a control tube, a finger, and a finger covered with repellent. As the results in Table 1 show, the reduction in response to the finger when it was covered with repellent was very small. Observation of individual flies showed that they frequently did not hesitate to probe and pierce even while their feet were placed in liquid 622. This is consistent with the idea that there are no areas or sense organs on the tarsi which are highly sensitive to stimulation by chemicals. Of a variety of compounds tested, including 1 M HCl, 1 M acetic acid, 5 M NaCl, liquid pentanol, and absolute methanol, not one prevented piercing. Individual flies behaved as though no chemical were present, and they fed to repletion. Flies in Bruce cages which were placed against a treated guinea pig remained away from the side of the cage touching the animal. If, in their random wandering, they alighted upon the side of the screen touching the animal, they immediately moved. This condition prevailed even when the feet of the fly came in contact only with the screen and not with the treated skin. Furthermore, flies without antennae, which normally failed to feed, showed little inclination to depart from the side of the cage against the treated guinea pig if they chanced to alight there.

Altogether these results suggest that the materials tested acted as olfactory rather than contact repellents but did not act at any great distance. That is, a fly was not prevented from approaching a feeding site, but upon coming close enough to land, either turned away or alighted only momentarily before departing. On the other hand, if, as in the case of the fixed specimens, the fly was unable to depart from the effective area of the repellent vapor, it responded to the attractive stimulus, heat, and fed through the repellent. It did, however, also regurgitate with greater frequency in the presence of a repellent, and this action suggests that regurgitation may be a characteristic response to noxious stimuli (as, for example, excessive heat) as well as a normal feeding response. It is com-

mon knowledge that other insects, especially caterpillars and grasshoppers, respond to repellents by increased salivation. The conclusions regarding the action of 622 are in agreement with the data obtained by other workers. Findlay *et al.* (1946), for example, have shown that 622 possesses some repellency for tsetses and that landing is reduced as much as 6 times, again suggesting a vapor action. The work of Sarkaria and Brown (1951) with mosquitoes also demonstrated the degree of vapor repellency of 622.

SUMMARY

The physiology of the biting responses of three species of tsetse flies was investigated. Specimens of *Glossina palpalis*, *G. morsitans*, and *G. brevipalpis* were attached by the wings to wax-tipped applicator sticks and subjected individually to a variety of stimuli. Their reactions were compared to those of free flying caged flies. Behavioral components of the normal biting response include characteristic movements of the mouthparts, salivation and defecation. Heat is the prime stimulus to feeding although contact of the feet with a substrate may alone elicit a low level of response provided the substrate is neither too smooth nor otherwise repellent. The antennae are the principal site of thermoreceptors mediating the feeding response. Neither odor nor chemicals in contact with the tarsi are effective stimuli. Responsiveness increases as the fly becomes more hungry, but as the final stages of starvation approach the fly becomes refractory. The repellent 622 prevents the feeding of caged free flying tsetses; however, attached flies will feed through this compound. 622 appears to act as an olfactory repellent.

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ON THE TOXICITY OF SCORPION VENOM[1, 2]

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The problem of scorpionism in Brazil has been extensively described by Campos (1924), Barros (1937), Magalhães and Guimarães (1940) and Magalhães (1946). The latter authority (1929) estimates the number of accidents by scorpions in Brazil as more than 6,000 annually with over 200 fatalities. In spite of the vast literature on clinical and experimental observations in this field, the opinions of the various authors on the amount of venom which can be ejected by a scorpion and on the toxicity of this venom are rather discordant. The solution of this question, however, is of great importance for the exact appreciation of the neutralizing and therapeutic capacities of specific antivenins as well as of the value of unspecific measures for the treatment of scorpion stings.

MATERIAL AND METHODS

The venom of *Tityus bahiensis* and *T. serrulatus*, the only two species of practical interest in Brazil, had been collected in the routine service of this Institute one day prior to performing the experiments. By means of mechanical stimulation the animals had been made to deposit their venom on a watchglass, where it had been dried. As the venom adhered very firmly to the surface of the watchglass, the latter was weighed and immersed in a suitable volume of physiological saline. After complete solution of the venom, the glass was taken out, repeatedly washed in saline and the washings added to the venom solution. The watchglass was then rinsed in distilled water, dried and once more weighed in order to determine by the difference between weights the amount of venom in the solution. The toxicity of the venoms was assayed in female albino mice by intravenous or subcutaneous injections in a volume of 0.02 ml. per gm. of body weight. Both routes of venom application were performed on the same day and using the same solutions and subdilutions in order to reduce as far as possible variations in the external influences on the animal response. Observation time was recorded until death or complete recovery ensued. Statistical evaluation of the results was done by probit analysis. Iterative calculations were continued until the expected probits obtained by the last cycle of computations did not differ by more than 0.01 from those of the previous one. Fiducial limits of the LD_{50} at $p = 0.05$ were calculated after formula (4.6) of Finney (1952). In one series of experiments, where the percentage mortality at successive dose levels was 0–0–0–0–33–100–100, the first 100 per cent figure was changed to 99 to make the probit method applicable.

[1] Carried out under Government of the State of São Paulo (Public Health) Contracts reg. nº TC 3861-50 and TC 47-53.

[2] Presented at the Staff Meeting of the Instituto Butantan, May 19, 1953.

RESULTS

Though the scorpions were kept and fed under rather uniform conditions there was a certain variation in the average individual venom yields on different dates of milking. Thus, 107 *T. bahiensis* ejected an average of 95 γ on November 28, 1951, whereas on February 14, 1952, 73 specimens, most of which were the same animals as before, gave only 38 γ each. Observations were similar with *T. serrulatus*, 170 of which yielded 76 γ per individual on January 7, 1952, but only 28 γ on the average in 124 specimens 22 days later.

The reactions to the venom inoculation in mice appeared immediately after injection by either of the routes of application and were the same as those minutely recorded in an excellent description by Maurano (1915), only that this author, who used venom extracted from triturated glands, did not observe the profound sweating manifest in all animals, even those which had received sublethal doses of venom. The most impressive feature of scorpion poisoning besides perspiration is the hyperexcitability of the intoxicated animals, which makes them assume defense positions similar to those of fighting males and wildly jump around on the slightest provocation. After lethal intravenous doses of *T. bahiensis* venom, death occurred in from less than 3 up to 20 minutes and after subcutaneous doses, from under 10 minutes to 3 hours. The corresponding figures for the toxin of *T. serrulatus* were 10 minutes to 3 hours (intravenous) and up to 6 hours (subcutaneous). It must be mentioned, however, that it was exceptional for a mouse which survived one to two hours after the venom injection to die later on. Dissection of the dead animals revealed general congestion but no macroscopically visible alterations that might have been caused by the venom. Local symptoms at the site of subcutaneous venom injections were absent.

The numerical results of the assays are given in Table 1. They demonstrate that there is no statistically significant difference in the toxicity of the two Brazilian scorpion venoms. It is further evident that the repetition of the experiments after a suitable interval with newly obtained venom, mostly from the same specimens as before, showed a markedly decreased toxicity in both of the cases. Most remarkable, however, is the fact that the LD_{50} of the two venoms is independent of the route of injection. Also the regression coefficients, 9.24 on the average, of all the mortality lines obtained in the present investigation were identical within the statistical limits of error. Thus, no experimental evidence was found that the physiologic mechanism of scorpion poisoning might be different with different parenteral ways of venom inoculation.

DISCUSSION

The average individual venom yields of the scorpions here recorded are partly in disagreement with the findings of other authors. Maurano (1915) reported a medium single yield of 1 to 1.5 mg. of dry venom on mechanical stimulation of *T. bahiensis*, whereas Magalhães (1928), by heat stimulation, obtained an average of 92 γ from 4,986 *T. bahiensis* and of 136 γ from 727 *T. serrulatus*, in which figures certain doubtful observations of that author are not included. The lowest average individual yields obtained by the latter author were 36 γ from

TABLE 1

SPECIES OF VENOM	ROUTE OF INJECTION	DATE	MICE					LOG-DOSE INTERVAL	LD_{50} mg./kg.	FIDUCIAL LIMITS AT p = 0.05	χ^2 (degrees of freedom)	g criterion
			Weight in gm.		Number							
			average	range	Total	Included in statistical evaluation	Per dose					
T. bahiensis	intraven.	29–11–51	18.1	16–21	47	18	6	~0.2	0.65	*	0.0 (1)	1.17
	subcutan.		17.2	15–20	90	40	10	~0.2	0.70	0.57–0.86	0.1 (2)	0.33
	intraven.	15–2–52	20.1	18–23	70		7	0.1	>2.00†			
	subcutan.		23.0	21–25	50		5	0.1	>2.00†			
T. serrulatus	intraven.	8–1–52	19.7	18–21	90	50	10	~0.2	0.49	0.40–0.60	0.7 (3)	0.22
	subcutan.		19.8	18–21	100	50	10	~0.2	0.64	0.53–0.74	0.3 (3)	0.23
	intraven.	30–1–52	21.1	18–23	99	40	10	0.1	0.77	0.69–0.86	2.6 (2)	0.27
	subcutan.		19.6	18–22	107		10	0.1	>1.60‡			

* Determination of fiducial limits by probit analysis impossible if $g \geq$ unity.
† No deaths occurred with doses $\leq$2.00 mgm./kg.
‡ Statistical evaluation not possible as highest dose tested gave only 30 per cent mortality.

140 *T. bahiensis* and 53 γ from 66 *T. serrulatus* presumably on first milking, which he considers more productive than later ones. So it looks rather probable that the estimate of Maurano (1915) is erroneously too high and that the real value lies in the region indicated by Magalhães (1928) and the present study.

There is a close analogy between the general symptoms of experimental scorpion poisoning in mice and those of scorpion sting in man according to the numerous case histories reported by the Brazilian authors quoted above. However, the profound sweating manifest in all the mice, even in many of those which had received venom doses far from the lethal ones, seems to be a less regular symptom of scorpionism in man. The absence of local reactions on intoxication by the scorpion species dealt with in the present paper is not characteristic for scorpion venoms in general; the venoms of other genera may provoke severe hemorrhagic edema at the site of inoculation, as reported by Cilli and Corazzi (1946).

The very short survival time even of the mice which had received lethal venom doses subcutaneously is in good agreement with the results of the few experiments on the same animal species recorded by Magalhães (1928) in the cases where he employed venom obtained from live scorpions. However, when that author used extracts from the triturated tissue of whole dissected venom glands, the mice did not die until more than 8 hours after the injection of fatal doses of such preparations. This is a proof that the process of venom extraction from ground glands, which by the way is the method most widely employed in institutions concerned with this matter, alters the properties of the venom considerably, thus rendering it unfit for research and antivenin assay. A second point against this method is the obvious impossibility of estimating even approximately the amount of venom in such an extract, though admittedly its physiological activity can be assayed biologically by comparison with a standard obtained from living scorpions in the more natural way. A simple consideration would suggest the abandonment of the extraction method also for venom to be used in antivenin production. There can be no doubt that the extract from triturated venom glands is highly contaminated with tissue juice, which, of proteic and thus antigenic nature, by the well-known concurrence of antigens (Benjamin and Witzinger, 1911; Lewis, 1915) may partly at least deflect the formation of antibodies in an undesired direction. Special investigations, however, are necessary before a definite opinion on this subject can be given.

The assay of the two toxins (Table 1) indicates that the toxicity of scorpion venom is well within the range of that of the tropical rattlesnake as recorded by Schöttler (1952). It is further evident that the toxic properties of scorpion venom are subject to considerable variation due to influences as yet unknown. Magalhães (1936) holds that Brazilian scorpion venoms are more active from August to October than between April and July,[3] but it is more than doubtful

[3] The original plan for the present study, to investigate the variations in the toxicity of scorpion venom on different milkings throughout the course of one year as well as to assay the therapeutic properties of specific antivenins, had to be given up due to an unforseen interruption in the venom supply, beyond the author's control.

that the experimental technique of that author justifies such conclusions. This is also the case with respect to his toxicity determinations (Magalhães, 1928), by which he established a "minimum lethal dose" of 9.57 mg./kg. for *T. bahiensis* from an experiment in 8 mice and of 3.25 mg./kg. for *T. serrulatus* from 7 animal experiments by what was described as the intramuscular route. He reported, for example, the injection of a volume of 2.0 ml. into a muscle of a 15 gm. mouse and even 1.0 ml. in a mouse of 5 gm., which on the face of it would seem to be an unlikely achievement.

At first sight, it might seem improbable that a small animal like the Brazilian scorpion, although gifted with a very potent venom, could kill an adult person. Indeed, the highest average of individual venom yields observed on large scale milking of scorpions was only 0.436 mg. in 211 specimens (Magalhães, 1928), but if the deviation of the maximum single yield from the average is as large in scorpions as in snakes (Schöttler, 1951) the amount of toxin which could be ejected by one animal may exceptionally attain to as much as 5 mg. Since the lowest dose of venom which, on subcutaneous administration, killed a mouse in the present experiments was approximately 0.4 mgm./kg., the quantity of poison at the disposal of one scorpion is sufficient to kill about 13 kg. of mice under a combination of unfavorable circumstances. Since considerable differences in the resistance of different species of laboratory animals to toxins are a well-known fact, the assumption that man is five times more susceptible to scorpion venom than the mouse is not far-fetched. It explains the rare fatal cases of scorpion sting in the adult and fully accounts for the markedly higher mortality in children. In the light of these considerations, reasonable volumes of scorpionic antivenin must neutralize at least 5 mg. of venom in order to be of therapeutic value.

All the reliable records on the toxicity of animal poisons show that there is an appreciable difference between the lethal doses of the same venom on subcutaneous and on intravenous administration, a ratio which is higher in the venoms with less neurotoxic action and lower in the toxins with a more pronounced activity on the nervous system. In a previous paper (Schöttler, 1952), this ratio was about 9 for the venom of the snake *Bothrops jararaca*, which causes circulatory collapse besides severe local gangrene, and 6 for the toxin of the tropical rattlesnake (*Crotalus terrificus*), which provokes mainly nervous paralysis. As shown in the present study the scorpion venom with an extremely violent action on the nervous system has a subcutaneous/intravenous ratio of unity, thus assuming a unique position among animal poisons. However, whether this ratio in a given venom is exclusively, or even really dependent on the degree of susceptibility of the nervous elements to this venom, and the mechanism of reaction by which this phenomenon is brought about, have not yet been ascertained. In the case of the scorpion venom, it is possible that the locus of attack is not central but peripheral and that the animals die from the continuous violent irritation of their nervous system together with the exhaustion resulting from the greatly increased muscular activity and from intoxication by the metabolites thus accumulated. Such an explanation is not contradictory to the behavior of the animals after

venom inoculation and is supported by the low subcutaneous/intravenous ratio of the respective $LLDD_{50}$. On the other hand, on the strength of extensive physiological and histological studies in combination with several hundreds of case reports, the principal reactions elicited by both scorpion venoms are ascribed to their action on the "bulbus" by Magalhães (1935), whereas Barros (1937) attributes them to a "selective toxicity for the central elements of the vegetative nervous system", which difference of opinion does not necessarily constitute a *contradictio in adjecto*, as the two opposing authors seem to believe. Their findings, however, are not incompatible with the explanation advanced above, as the detection of histopathological changes in organs taken after death is no conclusive proof as to the mechanism of the intoxication but is open to the criticism that the basic action of the venom has already occurred and the lesions encountered may be an effect rather than a cause of the metabolic derangement.

An interesting observation was made in connection with the statistical evaluation of the first assay recorded in Table 1, in which one of the observed 100 per cent responses was transformed into 99 per cent for computational purposes. From the standpoint of the biological experimenter this mortality curve, in which originally only one intermediate dose separated all the surviving animals from all the dead ones, will undoubtedly be considered as a very regular and close-cut response, an opinion also borne out by the low χ^2-test. However, statistical analysis of the variance of the regression coefficient reveals that the latter may also be negative, i.e., that the slope of the regression line may be reversed, indicating that animals which did not receive venom will die and that the chance of survival increases with increasing doses of venom. As the result of the empirical test is sound but that of the statistical evaluation is illogical, something must be wrong with the probit analysis. A discussion of this and other shortcomings of that method is beyond the scope of the present paper and will be treated on a broader basis elsewhere.

SUMMARY

The toxicity of the venoms of the Brazilian scorpions *Tityus bahiensis* and *T. serrulatus* is extremely variable at different times. The properties of venom extracted from triturated glands are different from those of the pure toxin obtained by milking the live scorpions. Mean lethal doses of from about 0.5 to more than 2.0 mg./kg. were found in the mouse test with the pure venom. There was no difference in toxic activity between the venoms of the two species. The LD_{50} was the same for subcutaneous and intravenous injection in both of the venoms. The latter observation in conjunction with the symptomatology of scorpion poisoning favors the assumption that the venom is mainly a peripherally attacking neurotoxin. Reasonable volumes of scorpion antivenin should neutralize at least 5 mg. of pure venom.

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THE SURVIVAL OF *BACTERIUM TULARENSE* IN LICE AND LOUSE FECES[1]

ROGER D. PRICE[2]

This paper presents the results of the first phase of a proposed study of the inter-relationship of the human body louse, *Pediculus humanus humanus* Linnaeus[3], and the tularemia organism, *Bacterium tularense*. The studies dealt with the determination of the longevity of *Bact. tularense* in lice killed immediately after infection, in infected lice allowed to starve to death, and in louse feces, each of the foregoing stored at various temperatures and relative humidities.

Hopla (unpublished, 1950) performed some preliminary experiments on body lice and tularemia, in which he found that infected lice maintained on normal rabbits retained the infection as long as three weeks and that unfed lice were still infected after six days, with a maximum infection of 27,000,000 organisms per louse.

MATERIALS AND METHODS

The body lice used were of the rabbit-adapted strain developed by Culpepper (1948) at the Orlando, Florida, laboratory of the Bureau of Entomology and Plant Quarantine, and were maintained essentially according to the methods recommended by that worker.

The *Bact. tularense* strain used throughout the study was the fully virulent Sm strain having an LD_{50} of $10^{-9.5}$ of a standard suspension for white mice (Downs and Woodward, 1949). A standard suspension of Sm organisms consisted of a 24-hour slant culture suspended in saline and adjusted to a turbidity of 24 per cent light transmission in the Coleman spectrophotometer at wave length of 600.

The agar used for the cultivation of this organism was glucose-cysteine blood agar (GCBA) prepared essentially after the method of Downs *et al.* (1947). This agar was modified slightly to contain a final 1:1,000,000 dilution of gentian violet. By preliminary experimentation it had been determined that this would reduce any possible gram-positive bacterial contamination but would not affect the growth of *Bact. tularense* in any abnormal manner.

The lice and feces were stored at four different temperatures as follows: 37°C., 29°C., 20°C., and 4°C. For each temperature they were stored at three different relative humidities (100, 50, and 0 per cent). The method of controlling

[1] This study is a contribution from the Departments of Entomology and Bacteriology, University of Kansas, Lawrence, and was supported by the General Research Funds of the University of Kansas.

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[3] Hopkins (1952) calls attention to this as the correct name of the louse.

the relative humidities was the sulfuric-acid method as described by Solomon (1951).

A method of louse infection following very closely the chick-skin technique described by Fuller *et al.* (1949) was used. In the preparation of the blood-Sm meal, an attempt was made to establish a meal containing as near 1 billion Sm organisms per ml. as possible. This was done by mixing 6.0 ml. of defibrinated normal rabbit blood with 2.0 ml. of a 10° Sm standard suspension. From the resulting 8.0 ml. of the mixture, 1.0 ml. was pipetted off and used for making serial ten-fold dilutions to 10^{-7}; duplicate plates of 0.1 ml. per plate were made at the 10^{-6} and 10^{-7} levels. This served as a check on the actual number of organisms present in each blood meal.

The removed chick skin was tied over the end of a glass tube, sealed with paraffin, and placed in a beaker containing the blood-Sm mixture and a few glass beads. Lice, consisting mostly of mixed adults starved 8–12 hours, were fed in groups of 75–100 for a period of 20–40 minutes, depending upon how readily they engorged. After feeding, the lice were removed to sterile distilled water, washed briefly for a total of 3–5 minutes in several changes of water, blotted dry on sterile toweling and treated thereafter according to the manner in which they were to be stored.

Small vials were used for all storage. The method of killing the lice consisted of touching each infected louse quickly on the head with a red-hot needle. These killed lice were stored five per vial at the different temperatures and relative humidities.

The method of collecting louse feces was determined by the subsequent conditions of storage. Feces were collected from 10 lice per vial for the 24-hour period immediately following infection. Lice were allowed to defecate for this period under the conditions of temperature and relative humidity in which the feces were to be stored except that, because lice will not defecate at 4°C., all collections for that temperature were made at 20°C. and the corresponding relative humidities. Two methods of fecal collection were employed. A special fecal collector was used to collect feces at 50 and 0 per cent relative humidity. It consisted of a piece of fine-mesh nylon gauze tied over one end of a short piece of glass tubing. This was suspended gauze-end down in a vial and infected lice were placed in the open end. The feces fell through the gauze to the vial floor. For collection of feces at 100 per cent relative humidity, a single-layered disk of bibulous paper was placed in the bottom of the vial and the lice were allowed to defecate directly on this.

The lice from which the feces had been collected were then starved. After the 24-hour defecation period, these lice were removed to new vials and stored 5 per vial under the same conditions at which they had defecated. Only at 4°C. was it necessary to use live lice that had not been allowed to defecate.

The combination of four temperatures and three relative humidities involved twelve different test situations. Under each of these twelve conditions at least 5 vials each of killed lice, starved lice, and louse feces were stored. This experiment, testing all 36 conditions, was gone through once and then completely repeated.

The only difference in the two lay in the fact that the first time there were 5 lice stored per vial with feces collected from 10 lice per vial, whereas in the second experiment 10 lice were stored per vial with feces being collected in the same manner as before.

For determination of the presence of *Bact. tularense* in the stored materials, a method of white-mouse inoculation was used. The material of each vial was ground as a unit in 1.5 ml. sterile normal saline with a sterile mortar and pestle. From this, 1.0 ml. was used to inoculate two mice intraperitoneally with 0.5 ml. each. This procedure was altered only on the day on which the lice were infected. Preliminary experiments on louse contamination had showed that only on this day could a reliable quantitative count be made on the Sm organisms in the lice. Thus, the lice on this day were ground in 2.5 ml. sterile saline. This was considered as a 10^{0} suspension and 1.0 ml. of it was withdrawn and used to make serial ten-fold dilutions to 10^{-4}. Duplicate GCBA plates with 0.1 ml. per plate were made for 10^{0}, 10^{-2}, and 10^{-4}. 1.0 ml. of the original suspension was used to make the usual mouse inoculations. Each mouse was observed twice daily and an autopsy made at the time of death if the mouse died within twelve days after the inoculation; mice surviving twelve days were sacrificed then and autopsied. Spleen and heart plate cultures were made for each mouse, and the lymph nodes, spleen, and liver were observed for gross evidence of tularemia. The plate cultures were observed after 24–48 hours for positive growth; if negative, they were allowed to incubate an extra 48 hours. The killed lice, starved lice, and louse feces were tested at varying intervals, depending on the observed trend of the survival of the organisms.

RESULTS

The main experiment involved eight separate preparations of the blood-Sm meal; preliminary experiments necessitated the preparation of four other of these meals; one control experiment required another. Thus, over all, there were thirteen separate chick-skin preparations and twelve infections of lice. In order to determine just how similar these infections were quantitatively, a duplicate plate count was made for the original blood-Sm meal and also for the newly infected lice. Plate counts were made possible for the lice only because the contamination encountered was diluted out at the 10^{-4} dilution, whereas the tularemia organisms were still present. Subsequent plate tests for organisms in stored lice met with consistent failure, due apparently to the increased contamination and decreased number of *Bact. tularense*. This contamination was not even eliminated when lice were treated with a variety of disinfectants before storage.

A comparison of the initial quantitative counts obtained in the thirteen blood-Sm preparations showed a relatively close similarity, the number of organisms ranging from a maximum of 1460×10^{6} per ml. to a minimum of 460×10^{6} per ml. with an average of 1045×10^{6} per ml. Initial louse infection showed a maximum of 2750×10^{3} organisms per louse and a minimum of 275×10^{3} organisms per louse, the average being 1151×10^{3} organisms per louse. Since it

TABLE 1

Longevity of Bacterium tularense *in killed lice, starved lice, louse feces, and blood on bibulous paper (in days)*

SUBSTRATE	RELATIVE HUMIDITY	EXPERIMENT 1				EXPERIMENT 2			
		37°	29°	20°	4°	37°	29°	20°	4°
	%								
Killed lice	100	4.0	4.0	7.5	22.0	3.0	5.5	17.5	25.5
	50	0.5	7.5	17.0	17.0	7.0	11.5	28.0*	29.0*
	0	2.0	14.5	17.0	41.0	4.0	17.0*	25.0*	45.5
Starved lice	100	2.5	6.5	8.0	28.0	3.0	5.0	14.0	22.5
	50	3.0	4.5	17.0	21.5	8.5	3.0	18.0	33.0*
	0	4.0	4.5	32.5	31.5	1.5	11.5	34.5	38.0*
Louse feces	100	1.5	4.0	10.5	20.5	2.0	1.0	3.5	23.0*
	50	9.5	7.0	17.0*	41.0	9.5	14.0*	26.0*	53.0*
	0	13.0*	20.0*	17.0*	50.0*	3.5	22.5	26.0*	53.0*
Blood on bibulous paper	100	No test made with Experiment 1				4.0	10.5	16.0	32.5
	50					0.5	0.5	24.5	49.0*
	0					0.5	0.5	9.0	32.5

* No final negative test.

was the original intent of this experiment to present the lice with a blood-meal containing 1 billion organisms per ml. in the anticipation of getting an average louse infection of 1 million organisms per louse, these quantitative counts showed these conditions were fairly well met.

The results of the main body of the work are given in Table 1. The data shown were arrived at by taking the mid-point between the last positive test and the first negative test. In the cases indicated by asterisks, where unfortunately no negative test was obtained after the last positive, two days were arbitrarily added to the last test. It was found in this respect that infection of lice and feces sometimes showed a very inconsistent tendency as the end-point was approached; occasionally one or two negative tests would be followed by a weakly positive test. Table 2 gives a comparison of the arithmetic and geometric means for the various main effects studied.

As a check to see if the lice were contributing anything to Sm longevity, the survival of *Bact. tularense* in blood alone was tested. The usual blood-Sm mixture was prepared and pieces of bibulous paper 5 by 10 mm. in size were dipped in this mixture and stored singly in vials under the various temperatures and relative humidities. According to Buxton (1947), a female louse starved 6–12 hours will take in approximately 1.0 mg. of blood. Because 10 lice were used per vial in experiment 2, it was assumed they would take in at most approximately 10 mg. of blood. This particular size of bibulous paper was found to absorb approximately 8–10 mg. of blood. The stored pieces of paper were tested at intervals in the same manner as the lice had been, each piece being ground in 1.5 ml. sterile saline and 0.5 ml. of this inoculated into each of two mice. A quantitative

TABLE 2

A comparison of the means of the main effects (in days)

MAIN EFFECT	ARITHMETIC MEAN	GEOMETRIC MEAN
Experiment 1	14.68	9.62
Experiment 2	18.00	11.57
Killed lice	15.52	10.33
Starved lice	14.83	9.75
Louse feces	18.67	11.66
37°C	4.55	3.45
29°C	9.08	7.05
20°C	18.66	16.38
4°C	33.05	31.06
100% rel. humid.	10.18	6.71
50% rel. humid.	16.79	11.74
0% rel. humid.	22.04	14.93

TABLE 3

Results of statistical analysis

SOURCE OF VARIANCE	d.f.	σ^2
Total	71	0.2104
Experiments (E)	1	0.1168
Humidities (H)	2	0.7613
Substrates (S)	2	0.0376
Temperatures (T)	3	3.1334***
E × H	2	0.1185
E × S	2	0.1266
E × T	3	0.0060
H × S	4	0.1985**
H × T	6	0.0666
S × T	6	0.0654
E × H × S	4	0.0130
E × H × T	6	0.0601
E × S × T	6	0.0206
H × S × T	12	0.0475
E × H × S × T	12	0.0517

Asterisks denote levels of significance:
*** $P < .001$.
** $.01 > P > .001$.

count at the time of absorption showed an initial count of 6 million organisms per piece of paper. The results of this experiment are shown in Table 1.

DISCUSSION

The data as presented in Table 1 were subjected to a four-factor analysis of variance, the factors tested being experiments (E), humidities (H), substrates

(S), and temperatures (T). A log transformation of the data was used after coding by multiplying each value by 10. The results of the analysis are shown in Table 3.

From this analysis it is readily seen that the factor of temperature was highly significant in contributing to the longevity of the organisms in the various substrates; the colder the temperature, the longer the *Bact. tularense* survived.

Humidities did not give a statistically significant difference. They did, however, show a trend in that direction, and, had a sufficient number of replicate tests been made, would presumably have reached the realm of significance. The trend lay in the direction of a longer survival at lower humidity, as evidenced by a mean survival of over twice the time at 0 per cent relative humidity as at 100 per cent humidity.

There was no significant difference shown among the three substrates or between the two experiments. The only other point at which significance appeared was in the humidity-substrate interaction. Undoubtedly the factor contributing most to this was the feces-humidity interaction. Feces, when collected at 100 per cent relative humidity, showed the shortest survival of all groups, whereas feces collected at the two lesser humidities showed the two longest survivals. This may have been due to the fact that feces in the dryer conditions dried very rapidly into small pieces and in this manner mechanically served as a form of protection to the tularemia organisms in the feces. Whatever the cause may be, it is interesting to note that consistently feces at the lower humidities, especially at 0 per cent relative humidity, showed the longest survival of the tularemia organisms. Along this same line, Francis and Lake (1922) found that the feces of tularemia-infected bedbugs, deposited on filter paper and subsequently dried, contained virulent organisms after 20 days, although they would be thought to be much more susceptible to desiccation and destruction in the tiny specks of dried feces than in the body of a killed louse.

When the length of survival of a starved louse is considered, some explanation can be obtained as to why survival of the organisms in them was little different from that in killed lice. According to Buxton (1947), starved lice at 30–35°C. will all die within 3 days and at as low as 10°C. all lice will die within 7 days. Half the lice will be dead usually in about half this time. Thus, essentially the only difference between starved lice and killed lice would be that the starved lice had had opportunity to void themselves of a considerable portion of the material in their digestive tract. This had no apparent effect in qualitative reduction of Sm longevity. Even if *Bact. tularense* were capable of maintaining itself in living lice, as the results of Hopla (1950) seemed to indicate, the starved lice did not survive long enough to make the difference evident. At the 4°C. temperature, starved lice ceased activity and became even more comparable to killed lice.

The saturated humidity created adverse conditions in that it was conducive to mold growth on the lice and feces at all temperatures but the lowest one. Even if the substrate escaped the mold, the high humidity made the substrate soft and spongy. Contrasted to this, survival of *Bact. tularense* in blood on paper was longer at saturated humidity at the two higher temperatures, with a tendency for the humidity effect to be negated at the lower temperatures. Lice and es-

pecially louse feces must have guarded the tularemia organisms against at least complete desiccation at these higher temperatures. The greatest point of contrast was found at 29°C. and 0 per cent relative humidity where the organisms on paper survived 0.5 days and in feces 20 and 22.5 days.

A glimpse into the quantitative number of organisms per louse was given by the initial counts per louse, a few successful subsequent counts on stored lice, and the average day of death (A.D.D.) of inoculated mice. These indicated an initial infection approximating 1 million organisms per louse; the rapidity of decrease after that was proportional to the temperature, the higher the temperature, the more rapid the decrease. The mouse A.D.D. often indicated that as the organism neared its end-point, organisms occurred in very small and inconsistent numbers. The whole trend was that of a dying off of *Bact. tularense* with environment determining the rate.

The implications of these experiments are quite clear. Lice under normal circumstances would probably undergo most of their existence within the temperature range of 20–30°C. and at a humidity predominantly lower than 100 per cent. This being true, if lice should have opportunity for a single infective meal from an individual suffering from tularemia, they would potentially be capable of spreading the disease for from 3–4 days up to several weeks even if they received no subsequent nutrition. The bodies of lice killed immediately after feeding or of lice starved to death, along with the feces of the latter, after lying unnoticed for a time, could accidentally come in contact with a suceptible individual and cause the disease. However, body lice in nature are host-specific to man, and until the time when the infectious dose of *Bact. tularense* for man is known and when adequate quantitative data are available for organisms in dead lice and feces, this method of louse infectivity to human beings will have to remain hypothetical.

SUMMARY AND CONCLUSIONS

1. Once a body louse, *P. humanus humanus*, became infected with *Bact. tularense*, both the louse and its feces harbored the tularemia organism for varying periods ranging up to 53 days, depending upon the environmental conditions.

2. Temperature was the greatest single contributing factor to longevity of the organism in the louse or louse feces, with lower temperature causing greater survival.

3. Humidity showed a tendency toward playing a significant part in survival, especially at higher temperatures; the lower the humidity, the longer was the survival.

4. No statistically significant difference was noted concerning longevity of *Bact. tularense* and the louse substrate. Louse feces, however, at low humidity, gave remarkable survival.

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RESULTS OF LABORATORY SCREENING TESTS OF CHEMICAL COMPOUNDS FOR MOLLUSCICIDAL ACTIVITY

II. Compounds of Mercury

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Since 1947 there has been under way in this Laboratory a screening program seeking compounds effective against *Australorbis glabratus*, an aquatic vector of *Schistosoma mansoni*. To date approximately 2000 compounds, principally organic, have been tested. The first paper in this series (Nolan, Bond and Mann, 1953) dealt with 701 compounds which were phenols or closely related substances. Over 200 compounds containing metallic elements have been tested. It is planned to report in several papers the screening results obtained with the metallic compounds; the present article deals with the results obtained with those containing mercury.

In 1920 Chandler reported that of various inorganic chemicals tested in the laboratory against *Lymnaea bulimoides* only mercuric chloride was comparable in efficiency to copper sulfate; he pointed out that the mercuric salt is not only more toxic to higher animals but is also more expensive. Mozley (1944) found that silver mercuric iodide was toxic to *Physopsis*. After the work in the present paper had been completed, there was brought to our attention an article by Szumlewicz and Kemp (1951) in which the high molluscicidal activity of phenylmercuric acetate was reported. McMullen (1952) listed diphenyl mercury and *p*-tolylmercuric chloride among the compounds which were found in laboratory tests to be moderately active against the amphibious snail, *Oncomelania nosophora*. We have tested 32 mercury compounds, including 6 mercurated phenols listed in Paper I of the series, and found them highly effective against *Australorbis glabratus*.

EXPERIMENTAL

The test procedures employed were fully discussed in Paper I of the series. The serial numbering of compounds used in this paper is a continuation of the numbers of that paper.

As before, 10 laboratory-reared *Australorbis glabratus* (5 snails/250 ml. of chemical solution) were used in each test. All chemicals were tested at 10 parts per million (p.p.m.) for a 24-hour exposure period followed by a recovery period of 24 hours, or longer if necessary, in fresh water with food provided. All of the mercury compounds were effective at 10 p.p.m. and were therefore tested at lower concentrations, *viz.*, 5, 3, 1, etc., p.p.m.

RESULTS AND DISCUSSION

The results obtained with the inorganic compounds of mercury are listed in Table I and the results with the organic compounds of mercury in Table 2.

TABLE 1

Results of screening tests with inorganic compounds of mercury

COMPOUND NUMBER	COMPOUND	MORTALITY OF *Australorbis glabratus* (PER CENT) AT:						
		10 p.p.m.	5 p.p.m.	3 p.p.m.	1 p.p.m.	0.5 p.p.m.	0.3 p.p.m.	0.1 p.p.m.
702	Mercuric acetate	100	90	100	10	—	—	—
703	Mercuric bromide	100	100	100	80	0	0	0
704	Mercuric chloride	100	90	100	30	0	0	0
705	Mercuric chromate	100	100	90	0	—	—	—
706	Mercuric nitrate	100	100	90	30	—	—	—
707	Mercuric oxalate	100	40	0	0	—	—	—
708	Mercuric sulfate	100	50	30	20	—	—	—
709	Mercuric thiocyanate	100	100	70	0	—	—	—
710	Mercuric cadmium iodide	100	90	100	10	—	—	—

TABLE 2

Results of screening tests with organic compounds of mercury

COMPOUND NUMBER	COMPOUND	MORTALITY OF *Australorbis glabratus* (PER CENT) AT:						
		10 p.p.m.	5 p.p.m.	3 p.p.m.	1 p.p.m.	0.5 p.p.m.	0.3 p.p.m.	0.1 p.p.m.
711	*n*-Propylmercuric thiocyanate	100	100	100	100	100	60	0
712	Isopropylmercuric thiocyanate	100	100	100	90	100	90	10
713	2-Hydroxypropylmercuric iodide	100	100	90	0	—	—	—
714	Phenylmercuric acetate	100	100	100	100	100	90	0
715	Phenylmercuric borate	100	100	100	100	80	30	0
716	Phenylmercuric bromide	100	100	100	100	60	10	0
717	Phenylmercuric chloride	100	100	100	100	70	30	0
718	Phenylmercuric gluconate	100	100	100	90	100	10	0
719	Phenylmercuric hydroxide	100	100	100	100	60	20	10
720	Phenylmercuric nitrate	100	100	100	100	90	60	0
721	Phenylmercuric salicylate	100	100	100	90	70	30	0
722	Sodium ethylmercurithiosalicylate ("Merthiolate")	100	0	0	0	—	—	—
723	Dibenzyl mercury	100	100	100	100	50	0	0
724	8-Phenylmercurihydroxyquinoline	100	100	100	100	70	30	0
725	2-Chloromercurifuran	100	100	100	100	90	30	0
726	Diethylmercuric perthiocyanate	90	80	100	0	—	—	—
727	(2,3-Dimethoxytetramethylene)bis-mercuric acetate	100	100	60	0	—	—	—
619	2-Hydroxyphenylmercuric chloride	100	100	100	60	40	0	0
620	4-Hydroxyphenylmercuric chloride	100	100	100	60	0	0	0
621	2,4-Dichloromercuric phenol	100	100	100	40	60	0	0
622	4-Hydroxyphenylmercuric thiocyanate	100	100	100	60	—	—	—
623	4-Nitro-3-hydroxymercuri-*o*-cresol anhydride ("Metaphen")	100	100	90	0	—	—	—
624	Disodium dibromohydroxymercurifluorescein ("Mercurochrome")	90	0	0	0	—	—	—
90	Pentachlorophenol	100	100	100	10	0	0	0

Included in the latter are the data obtained with the mercurated phenols previously reported. The data obtained with pentachlorophenol are also included to facilitate comparison.

There has been omitted from the table an organic compound of somewhat doubtful structure with a low mercury content, No. 728, the "mercury (I) salt of the monobutyl ester of α-alkenyl-succinic acid," $RCHCOOCH_2(CH_2)_2CH_3$. CH_2COOHg

This compound had been reported as exhibiting fungicidal properties. In screening tests it was inactive at 10 p.p.m. against *Australorbis glabratus*.

It is interesting that with the mercurials as well as with the effective phenolic compounds (Table 1, Paper I of the series) there is on the whole a high molluscicidal potency above a given concentration, followed by a rapid loss of efficiency upon dilution. This is in marked agreement with the bactericidal or bacteriostatic action of certain phenols.

There appears to be no correlation between the mercury content and the activity of a compound. Two of the compounds with comparatively low mercury content, mercuric cadmium iodide (24.3% Hg) and mercurochrome (26.7% Hg), are among the least effective compounds; on the other hand, mercuric oxalate (69.3% Hg) and mercuric sulfate (67.6% Hg) likewise are of low molluscicidal activity. The mercury composition of each of the remaining 28 compounds lies within the range of 42.4–73.9 per cent.

The phenylmercuric salts are of a high order of molluscicidal efficiency. They are also known to possess high bactericidal and fungicidal power. The organic mercurials in general have been considered to be less irritating and less toxic to higher forms of life than the inorganic mercury compounds. Weed and Ecker (1931) reported that phenylmercuric nitrate, for instance, is relatively nontoxic, whether given orally, intraperitoneally or subcutaneously to laboratory animals. However, phenylmercuric acetate when administered in the diet of the rat has been shown (Fitzhugh *et al.*, 1950) to be chronically more toxic than mercuric acetate.

It will be noted that many of the organic mercurials (Nos. 711, 712, 714, 715, 716, 717, 718, 719, 720, 721, 723, 724, and 725) are still fully effective at 1 p.p.m., and a few (Nos. 711, 712, 714, 718, 720, and 725) even at 0.5 p.p.m. Based on results at 0.3 p.p.m., isopropylmercuric thiocyanate (No. 712) and phenylmercuric acetate (No. 714), each with a 90 per cent kill, as well as *n*-propylmercuric thiocyanate (No. 711) and phenylmercuric nitrate (No. 720), each with a 60 per cent kill, are the most potent molluscicidal compounds of mercury which we have tested. Indeed, many of the mercury compounds are the most effective molluscicides so far found. They are more effective in the laboratory than pentachlorophenol, which is the "compound of choice" at present, based on availability, cost, reliable mammalian toxicity data and field performance. Most certainly, the mercury compounds should be thoroughly investigated in the field since, although they are known to be toxic to man and other animals, the concentrations necessary to effect a rapid and complete kill of snails are so low that these compounds perhaps can be applied with safety to waters used by man.

SUMMARY

Results of laboratory tests with 32 inorganic and organic compounds of mercury are presented. Many of the mercury compounds constitute the most effective molluscicides so far reported.

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DISINSECTIZATION OF AIRCRAFT BY RESIDUAL DEPOSITS OF INSECTICIDES

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It is necessary to control arthropod vectors of diseases that are accidentally transported in aircraft so that dangerous species will not be introduced into new areas and infected insects will not set up new foci of infection. Methods of disinsecting aircraft that are at present acceptable to responsible health authorities involve the use of pyrethrum aerosols, sometimes supplemented by residual deposits of DDT (Duquet, 1949; WHO, 1951). Although these aerosols are effective against mosquitoes (*Anopheles quadrimaculatus* and *Aedes aegypti*) in passenger compartments of airplanes, they are not effective against free-flying house flies (*Musca domestica*) in these compartments when dispensed at the recommended dosages while the planes are in flight (Maier *et al.*, 1953). Also, larger than the recommended dosages of these aerosols were required to give a satisfactory percentage of mortality of free-flying house flies and mosquitoes released in baggage compartments of planes.

This study on residual insecticides for disinsecting aircraft was undertaken to determine if such materials might be of value as supplements to the routine aerosol treatments or if some of them might be worth consideration as replacements for aerosols.

Procedures. Through the generous cooperation of the Caribbean Atlantic Airlines, Inc., the Eastern Air Lines, Inc., and the Pan American World Airways, Inc., it was possible to test residual insecticides in aircraft operating on routine schedules. Insects were exposed under realistic free-flight conditions in both airborne and grounded DC-3 and Convair-240 planes. All of the interior surfaces of the baggage compartments and all surfaces in passenger cabins except those changed routinely, such as slip covers and pillows, were sprayed with solutions of insecticides in methylcyclohexane. Preliminary tests had shown that this solvent would not damage plexiglas or other aircraft materials. An ordinary paint sprayer was used to apply the solutions. Later this equipment was replaced by a modified hand-type pneumatic pressure sprayer in an attempt to find a way to apply a more uniform residue of insecticides in passenger cabins.

After treatment, each baggage compartment was loaded to capacity with sealed empty cardboard boxes to simulate luggage and parcels. These boxes were either replaced or recovered after every three tests. Before a plane was used for a second treatment it was carefully cleaned with solvent to remove any insecticides that might have remained from previous applications.

About 200 mosquitoes (*A. aegypti*), and 200 female house flies (*M. domestica*) of the NAIDM strain were released in the treated compartments. They were permitted free selection of resting surfaces, including the untreated boxes in

TABLE 1

Twenty-four-hour mortalities of free-flying female house flies (Musca domestica) *and mosquitoes* (Aedes aegypti) *exposed to residual deposits of insecticides in baggage and passenger compartments of DC-3 and Convair-240 aircraft at San Juan, Puerto Rico, 1952–53*

INSECTICIDE	RESIDUE (mg. per sq. ft.)	TEST SITE AND DURATION	TYPE COMPARTMENT	NUMBER PLANES TREATED	24-HR. MORTALITIES (%) OF TEST INSECTS BY WKS. AFTER TREATMENT															
					House flies (*Musca domestica*)								Mosquitoes (*Aedes aegypti*)							
					1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
DDT	200	In-flight (90 min.)	Baggage	2	75	69	72	54	57	—	—	—	75	45	55	34	18	—	—	—
Dieldrin	100	On-ground (90 min.)	Passenger	1	98	97	92	—	—	—	—	—	—	—	—	—	—	—	—	—
Lindane	200	In-flight (90 min.)	Baggage	1	100	100	100	99	99	81	90	64	100	100	100	100	100	100	86	85
Lindane	200	On-ground (90 min.)	Baggage	11	100	100	100	99	95	89	90	77	100	100	100	100	100	94	92	83
Lindane	200	On-ground (45 min.)	Baggage	2	100	100	100	99	100	93	92	—	100	100	100	100	100	100	100	—
Lindane	100	On-ground (90 min.)	Baggage	10	100	94	—	—	—	—	—	—	100	100	—	—	—	—	—	—
Lindane	100	On-ground (90 min.)	Passenger	3	99	97	73	—	—	—	—	—	100	100	98	—	—	—	—	—
Lindane	70	On-ground (90 min.)	Passenger	3	100	99	100	82	86	74	—	—	100	100	100	100	100	—	—	—
Lindane	50	On-ground (90 min.)	Passenger	8	99	76	90	—	—	—	—	—	100	98	100	—	—	—	—	—
Lindane-Aroclor*	200–200	On-ground (45 min.)	Baggage	2	96	100	85	98	80	87	82	—	—	—	—	—	—	—	—	—
Lindane-Aroclor*	200–67	On-ground (45 min.)	Baggage	2	98	75	80	—	—	—	—	—	—	—	—	—	—	—	—	—

* Aroclor-5460 is a mixture of chlorinated biphenyl and chlorinated poly-phenyls manufactured by Monsanto Chemical Co., St. Louis, Mo.

the baggage compartment tests, and were recaptured after either 45-minute or 90-minute exposure periods. Flies were tagged with 1-inch-long colored nylon threads glued to the thorax (Klock *et al.*, 1953) to facilitate their recapture and to aid in identification of the ones used in each separate test in passenger or baggage compartments. Satisfactory percentage of kill of the test insects was arbitrarily set at a level of 95 per cent mortality of the house flies that were recovered and held under laboratory conditions for 24 hours.

Results of the tests. Results of these field tests are tabulated in Table 1. They show that DDT applied to baggage compartments at 200 mg. per sq. ft. did not produce satisfactory kills of either flies or mosquitoes. Based on treatment of one plane with tests made on the ground, dieldrin gave satisfactory house fly mortalities for two weeks after it was applied to the passenger cabin. Its effectiveness was reduced by the third week.

Lindane showed considerable promise of giving satisfactory mortality of flies and mosquitoes in both passenger and baggage compartments. Five weeks of satisfactory kills of house flies were obtained in baggage compartments containing 200 mg. per sq. ft. residues. Only about one week of satisfactory fly mortality was obtained with a treatment of 100 mg. lindane per sq. ft. in baggage compartments. In these limited tests there appeared to be no difference in results when tests were made "in-flight" or "on the ground;" also, 45-minute exposures were about as effective as 90-minute exposures. All test lindane treatments were effective for longer periods of time against mosquitoes than against house flies.

Lindane produced objectionable spotted surfaces in passenger compartments when applied at 200 mg. per sq. ft., so preliminary tests were made to determine the effectiveness of lighter dosages which did not cause spotting. When applied at 50 and 70 mg. per sq. ft. it gave satisfactory mortality of test insects for one and three weeks, respectively. It is noted that 70 mg. residues were effective longer than 100 mg. residues. Although the reasons for this are not known, they probably involve variations in the uniformity of deposition that are inherent in the methods used to apply these lower dosages of the material. Development of other techniques for applying lindane and determining the maximum permissible dosage in passenger cabins is needed.

In the limited number of tests made in which a resin (Aroclor-5460) was added to lindane in an attempt to extend its period of residual effectiveness, the periods of satisfactory fly kills were considerably reduced.

Although residual deposits of lindane appear to show promise for disinsecting aircraft, mammalian toxicological studies are needed to evaluate the hazards to passengers and crew members that may be connected with vapors from the residues before this method of disinsectization can be adopted for general use.

SUMMARY

Field tests with free-flying house flies and mosquitoes in passenger aircraft that were being used on routine schedules have shown that lindane residues of 200 mg. per sq. ft. will satisfactorily disinsect baggage compartments for five weeks with a single application. These dosages are objectionable in passenger

cabins because of spotting, but lindane residues of 50 and 70 mg. per sq. ft. are not objectionable in this respect and these were effective in the cabins for one and three weeks, respectively. DDT and dieldrin residues applied at reasonable dosages were found to be inferior to lindane deposits for aircraft disinsectization.

Further studies on application methods for lindane and on the toxic hazards to passengers and crews of the vapors from lindane residues must precede general use of this method for aircraft disinsectization.

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A SIMPLE TECHNIC FOR OBTAINING EGG AND SERUM SLANTS FREE OF BUBBLES[1]

NORMAN D. LEVINE AND WILLIAM C. MARQUARDT

College of Veterinary Medicine and Agricultural Experiment Station, University of Illinois, Urbana, Illinois

A common culture medium for many species of parasitic protozoa is a coagulated egg slant overlaid with a nutrient solution. Serum slants are sometimes substituted for the egg slants. Serum and egg slants are also often used for the cultivation of bacteria. In preparing the slants, special measures must be taken during the sterilization process to prevent bubbles from forming and destroying their smooth surface. Several methods have been used. Most involve inspissation at a relatively low temperature followed by sterilization. One method mentioned by Levine and Schoenlein (1930) consists of slanting the tubes, heating them slowly to 75–80°C., holding them at that temperature for an hour to inspissate, and then reheating for an hour on each of two successive days to complete sterilization. In a second method the tubes are placed on a slant in an autoclave and the temperature is raised slowly to 80–85°C. by allowing steam to enter but not allowing the air to escape. After the slants have coagulated, the air is replaced by steam, and the tubes are sterilized under pressure. After sterilization, the pressure is lowered slowly. Craig (1948) recommended a very similar technic, suggesting that egg slants be inspissated at 70°C. and then autoclaved at 15 pounds pressure for 20 minutes. Foster and Cohn (1945) insulated the tubes by placing them in gallon mayonnaise jars lined with cotton, while Spray and Johnson (1946) reported a method using a preheated autoclave.

In other methods, air is kept in the slants. Howitt (1925) autoclaved egg slants at 15 pounds pressure for 20 minutes in an autoclave from which the air was not allowed to escape. Hinkleman (1923) devised a metal rack which held corks tightly in the culture tubes during autoclaving. Coagulation and sterilization were completed in 30 minutes at 10 pounds pressure.

The various methods suggested for obtaining smooth slants act by minimizing the formation of bubbles. Slow heating and relatively low temperatures permit the air to escape slowly and in small bubbles. Corking the tubes keeps the air pressure up so that no air leaves the slants.

A technic developed in this laboratory involves neither intermittent heating nor special manipulation of the autoclave, and produces smooth slants. By its use, the air is removed before the medium has been coagulated, leaving practically none to come out during subsequent autoclaving. This method has given more uniformly satisfactory results than other methods which were previously used. It was first developed for egg slants, but has also been used successfully in making serum slants.

In preparing the egg slants, 12.5 ml. of Ringer's solution are mixed thoroughly

[1] This investigation was supported by a research grant (E387) from the National Institutes of Health, Public Health Service.

with each egg used. For best results, the mixing is performed in a Waring blendor for 30 seconds. If the Waring blendor is not used, bubbles may form occasionally, particularly in serum slants, but when it is used bubble formation is rare. If the blendor is not used, the mixture should be filtered through cheesecloth. Two milliliter amounts are then pipetted into 18 x 150 mm. cotton stoppered tubes, and the tubes are placed upright in a vacuum desiccator. The desiccator is evacuated slowly. As evacuation proceeds, the egg mixture begins to bubble, and within four minutes a dense foam of egg begins to climb in the tubes. Evacuation is stopped before the cotton plugs become wet, and the tubes are allowed to remain in the evacuated desiccator for an hour. The vacuum is then released, the tubes are packed in baskets, slanted in the autoclave, and inspissated and sterilized like other media at 15 pounds pressure for 20 minutes.

Best results are obtained when no butt of medium is left in the tubes. When this is done, 2 ml. of fluid makes a slant about 1½ inches long in an 18 x 150 mm. tube.

In normal operation of the autoclave, air dissolved in the slants is pulled out of them after the air in the chamber has been replaced by steam. Since the slants are coagulated when the air bubbles leave them, their surface is ruptured. They are too soft to prevent the air from escaping in large bubbles, and too firm to flow back into a smooth surface after the bubbles have broken it.

The presence of steam under pressure in the autoclave has no effect on the rate at which air diffuses out of the slants, since this depends on the partial pressure of the air alone. If there is no air in the autoclave, the air in the slants leaves them at the same rate that it would in a vacuum.

The use of a vacuum to remove dissolved air in the preparation of egg and serum slants seems to be a simple and logical step. However, since we have not encountered reference to it either in periodical literature or in standard texts, we feel it should be recorded.

SUMMARY

A technic is described for obtaining smooth-surfaced egg or serum slants by removing the air dissolved in them under vacuum while they are still liquid. The tubes are then slanted in the autoclave, and the media coagulated and sterilized without special care at 15 pounds pressure for 20 minutes.

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CORRESPONDENCE

Indian Medical Gazette
School of Tropical Medicine
Chittaranjan Avenue
Calcutta, India

May 12, 1953

To the Editor:

In the Indian Journal of Medical Research Vol. 10, 1922–23, p. 943, you will find described by me, from the Independent Brigade Laboratory of Aden, *Schistosoma haematobium* and *S. mansoni*, occurring in the same part of Arabia as has been surveyed recently by the U. S. Naval Medical Research Unit No. 3, Cairo, Egypt (Robert E. Kuntz, George M. Malakatis, Deaner K. Lawless and Cloyce P. A. Strome, your journal, Vol. 2, p. 13; January, 1953).

Incidentally, I then (1923) expected that in all diagnoses of neoplasm in the bladder or rectum, schistosomes would be excluded. About 8 years later, however, when I was posted at the Haffkine Institute, Bombay, I received a specimen from Aden for confirming cancer. The patient (a princess) had been operated upon and had died. A heavy concentration of the schistosome ova was found in the tissue received.

Obviously my findings have been ignored by clinicians and laboratory workers alike: hence this note.

Yours truly,
S. D. S. Greval
Editor, Indian Medical Gazette

CORRESPONDENCE

September 4, 1953

To the Editor:

In reference to the article by Drs. Carlos Calero M. and Carl M. Johnson on "Cutaneous leishmaniasis in the Republic of Panama" in No. 4 (July) 1953 permit me to add the following information:

Cutaneous leishmaniasis is rather common in the Province of Bocas del Toro of Panama and in the adjacent area of Costa Rica. From 1944 up to the present, involvement of the *nasal mucosa* has been observed here in eight cases. One of these I reported as "Probable Polypous Form of Leishmaniasis of the Nose" in *Practica Oto-rhinolaryngologica* **14:** 14 (1952). The patient returned later-on when antigen for the intracutaneous Montenegro test was available. The test resulted strongly positive; antimony therapy was again successful and thus the diagnosis was beyond doubt. Description of the other cases of nasal leishmaniasis will be published elsewhere.

In mucosal lesions the parasites are rarely encountered. The histological find-

ings are not fully characteristic, but for the experienced pathologist it is often possible to express suspicion of leishmaniasis. In establishing the diagnosis the Montenegro test is very helpful and the success of the antimony treatment clinches the diagnosis after syphilis, yaws, scleroma, malignancy, etc. have been excluded.

Yours very truly,
Ludwig Jaffé, M.D.

BOOKS RECEIVED

Respiratory Diseases and Allergy. New Method of Approach, by Josef S. Smul, M.D., Fellow National Gastro-Ent. Assoc., Member N. Y. Academy of Sciences. 80 pp. New York 3, New York, Medical Library Co., 1953. Price $2.75.

The Anopheline Mosquitoes of the Indo-Australian Region, by J. Bonne-Wepster, M.D., in collab. with N. H. Swellengrebel, Ph.D. from the Department of Tropical Hygiene and Geographical Pathology of the Royal Tropical Institute, Amsterdam. 504 pp., illustrated. Amsterdam, J. H. de Bussy, 1953.

Symposium on Stress (16–18 March 1953). Army Medical Service Graduate School and Walter Reed Army Medical Center. 332 pp. Washington 25, D. C., U. S. Government Printing Office, May 1953. Price $.60.

Acute Infectious Hemorrhagic Fevers and Mycotoxicoses in the Union of Soviet Socialist Republics. Army Medical Service Graduate School and Walter Reed Army Medical Center. 140 pp. Washington 25, D. C., U. S. Government Printing Office, May 1953. Price $.60.

A Bibliography of Toxoplasmosis and Toxoplasma gondii, by Don E. Eyles, Sc.D., National Microbiological Institute and Jacob Karl Frenkel, M.D., Ph.D., National Cancer Institute. 47 pp. Public Health Service Pub. No. 247, Washington 25, D. C., U. S. Government Printing Office, 1952. Price $.20.

Synopsis of Medical Entomology, by V. E. Brown, Ph.D., Marquette University. 219 pp., illustrated. Ann Arbor, Michigan, Edwards Bros. Inc., 1953. Price $4.50.

Synopsis of Medical Parasitology, by V. E. Brown, Ph.D., of Marquette University. 107 pp., illustrated. Milwaukee, Wisconsin, V. E. Brown, 1953. Price $3.75.

Expert Committee on Plague, Second Report. WHO Technical Report Series No. 74. 13 pp. Geneva, WHO. Distributed by Columbia University Press, International Documents Service, 2960 Broadway, New York 27, N. Y. 1953. Available also in French edition. Price $.10, 9 d., fr. Fr. 30, Sw. fr. 0.40.

Ten Lepers from Naestved in Denmark. A study of skeletons from a medieval Danish Leper Hospital, by Vilhelm Moller-Christensen, M.D. 160 pp., illustrated. Copenhagen, Danish Science Press, Ltd., 1953.

Design and Operation of Septic Tanks. WHO Monograph Series No. 18, 124 pp., illustrated. Geneva, WHO. Distributed by Columbia University Press, International Documents Service, 2960 Broadway, New York 27, N. Y. French edition in preparation. Price $1.50, 7/6, Fr. fr. 480, Sw. fr. 6.—.

Difco Manual, Ninth Edition. Difco Laboratories, Inc. 35 pp. Detroit 1, Michigan, Difco Laboratories, Inc. 1953.

World Directory of Medical Schools. WHO; clothbound, 220 pp. Geneva, WHO. Distributed by Columbia University Press, International Documents Service, 2960 Broadway, New York 27, N. Y., 1953. Bi-lingual edition, Eng.-Fr. Price $4., 25/-, Fr. fr. 1,200, Sw. fr. 15.—.

Expert Committee on Leprosy. First Report. 1. Epidemiology. 2. Control. 3. Treatment. 4. Classification. 5. Immunology. 6. Significance of histopathological examinations. WHO Technical Report Series No. 71. 28 pp. Geneva, WHO, 1953. Available also in Fr. ed.

Dist. by Columbia University Press, International Documents Service, 2960 Broadway, New York 27, N. Y. Price $.20, 1/6, Fr. fr. 65, Sw. fr. 0.80.

Joint UN/WHO Meeting of Experts on the Mental-Health Aspects of Adoption. Final Report. WHO Technical Report Series No. 70. 19 pp. Geneva, WHO, 1953. Available also in French edition. Distributed by Columbia University Press, International Documents Service, 2960 Broadway, New York 27, N. Y. Price $.15, 1/3, Fr. fr. 50, Sw. fr. 0.60.

Expert Committee on Venereal Infections and Treponematoses, Fourth Report. WHO Technical Report Series #63. 84 pp., illustrated. Geneva, WHO, 1953. International Documents Service, Columbia University Press, 2960 Broadway, New York 27, N. Y. Price $.55, 4/3, Sw. fr. 2.20.

Parasitology (1949–1950), by Juan Enrique Mackinnon, Facultad de Medicina, Universidad de Montevideo, Uruguay, in collaboration with Sammuel B. Pessôa, Faculdade de Medicina da Universidade de São Paulo, Brasil; Felix Pifano, Facultad de la Universidad Central de Venezuela, Caracas and Alfonso Trejos, Hospital San Juan de Dios, San José-Costa Rica. 287 pp. w. bibliography of 36 pp. UNESCO Science Cooperative Office for Latin America, Montevideo, 1953.

BOOK REVIEWS

The Natural History of Infectious Disease by Sir Macfarlane Burnet, M.D., F.R.S., Director of the Walter and Eliza Hall Institute, Melbourne, Australia. 356 pp. with illustrations. New York: Cambridge University Press. 1953. Price $4.50.

The book is a second edition of an earlier publication "Biological Aspects of Infectious Disease," with a new title and a sound account for all that has happened since 1940 toward an improved and practical approach to problems of acute infection. These things are many; astonishingly so, as developments are traced through antibiotics and chemotherapy to insectides and insect repellents; and not neglecting the growing appreciation of a social component in mass infection.

The admirable framework of the first edition has been maintained; it would be a pity had it been disturbed. The first two chapters deal with the ecologic point of view and with the evolution of ideas of infection and defense. A final chapter treats of new diseases and the outlook for the future. Of themselves these three chapters are enough to make any book worthwhile. In between is discussion of infectious agents and of defense mechanisms, then a consideration of mass disease of human populations where natural history guides interpretation of modes of spread, endemic and epidemic prevalence, and gives a basis for control measures. Some 70 pages are devoted to illustration of the principles defined, primarily through the great quarantinable diseases of man—plague, cholera and yellow fever—but not forgetting such everyday matters as diptheria and tuberculosis. The theme is expressed promptly, on page 4, that "the application of the ecologic point of view to the problem of infectious disease probably represents the most important change of attitude by microbiologists in the past 20 or 30 years."

The book is anything but a technical treatise on the elements which enter into infection. The biologist will find a presentation of fundamental factors as they fit into the general scheme of living things. The scientific generalist will see biology in terms of human affairs; and for the medical student infection becomes something more than a clinical phenomenon. A forthright presentation in warm intimate style, the book is not written down to anyone's level. The intelligent layman can follow without difficulty, the specialist in microbiology and epidemiology will meet all manner of fresh interpretation of old problems.

Regrettably the proof reading is careless; the book will be read widely and by many foreigners to the English language (infect for insect; P. falaparum twice for P. falciparum). Some microbiological bias is to be expected; that is the author's field and also one of the reasons for preferring the old title. Nevertheless, in down-to-earth fashion and within a

complicated area many disciplines are brought together, giving emphasis to the need for the unifying science which is ecology. This is no ordinary book, perhaps having most in common with Martini's *Wege der Seuchen*.

JOHN E. GORDON

African Highway—The Battle for Health in Central Africa by SIR MALCOLM WATSON, M.D., LL. D., Hon. Consultant, Ross Institute of Tropical Hygiene, London, with an introduction by Sir Eric Macfadyen. 294 pp. with illustrations and maps. London: John Murray. 1953 Price 3/3.

This is a remarkable book. Into it Sir Malcolm Watson has distilled a half century of experience in tropical sanitation, and he presents a philosophy refreshingly different from that usually adopted in relation to disease control in the tropics and among relatively primitive people.

A large part of the book is devoted to malaria control, but other diseases in central Africa are discussed. Basically, if malaria is controlled on the one hand, and the enteric infections are controlled on the other, a long step has been taken toward a reasonably healthful existence for both the white and colored races in the tropics.

The book is also important in its consideration of the relation of health to the economy of the area, in its understanding that sanitation must be adapted not only to climate and other environmental factors, but also to the mores and customs of the people affected. Its constant attention to the ecology of the local mosquito species in relation to control measures is also most valuable.

The book will be of great value to every one concerned with health in the tropics. The highway to health in the tropics exists because men like Watson first made a path.

HAROLD F. GRAY

Dermatology in General Practice by JACOB HYAMS SWARTZ, M.D., Assistant Professor of Dermatology, Harvard Medical School, Consulting Dermatologist, Massachusetts General Hospital; with a foreword by C. Guy Lane, M.D., Professor Emeritus, Department of Dermatology, Harvard Medical School. 581 pp. 253 illustrations. Baltimore: The Williams & Wilkins Co. 1953. $11.00.

This book presents a pleasant deviation from the conventional arrangement of similar dermatological works in as much as in addition to anatomy and physiology, detailed valuable information is given regarding the care of normal and abnormal skin. The physician is often asked by his patients for advice regarding care of face, scalp, hair, feet, exposure to sun and similar problems. This book gives such advice. Very useful, also, are the tables for the differential diagnosis of common skin disorders, the numerous well chosen photographs as well as the expertly written chapter on fungous disease. The microphotographs and histopathological comments by Lever are excellent.

It is understandable that such a compact book can not cover uncommon diseases in detail. However, statements like "Leprosy is chronic, contagious and infectious. . . . The disease terminates fatally," can be misleading if not amplified. Also, the classification of leprosy does not reflect the latest views.

The therapeutic advice in general is excellent, especially in the important chapters on acne, psoriasis, eczema and impetigo, and valuable details are given regarding the technic of application of the prescribed medication including baths, dressings, compresses etc. There might be objection to the recommendation of carbolic acid shampoo for pediculosis, without stressing the contraindications and especially establishing an age limit. The same applies to Asterol in the treatment of fungous diseases. Among scalp lesions metastatic tumors might deserve mention.

The above suggestions and disagreements are not intended to detract from the great value of this book.

PAUL FASAL

First International Symposium on Yaws Control, Bangkok, 1952. WHO Monograph Series No. 15. Mixed language edition. 418 pp., 32 plates. WHO, Geneva. Distributed by Columbia University Press, International Documents Service, 2960 Broadway, New York 27, New York. Price $4.50; Fr. fr. 1,440; Sw. fr. 18.

A world map of the geographical distribution of yaws shows this hideous and disabling infection to be coextensive with the tropics around the world. Two circumstances which happened to coincide in time have brought the disease into prominent notice in recent years: the development of an effective and relatively inexpensive therapy with antibiotics, which almost realizes Ehrlich's dream of a single-injection cure, and the strong impulse given to international health work by the creation of the WHO. This symposium, which consists of 18 papers running to 392 pages and abundantly illustrated, covers in a methodical way our present knowledge of yaws—its biology, epidemiology, treatment and control—with examples drawn from experience in the tropics of South East Asia, Africa and the Americas. There are still many problems: its exact relationship to syphilis; the role of insects in transmission; classification of latent stages; the optimum treatment schedule; and the best measures of control. This monograph does not pretend to give all the answers, but it presents the opinions and experiences of yaws workers from all over the world.

L. W. HACKETT

Insecticides. Manual of Specifications for Insecticides and for Spraying and Dusting Apparatus. Loose leaf binding, to be supplemented through December 31, 1955. WHO, Geneva, distributed by International Documents Service, Columbia University Press, 2960 Broadway, New York 27, New York. Price $8.50, £3, Fr. fr. 2800, Sw. fr. 35.

This is a compilation, in a substantial loose leaf binder, of the various specifications which have been prepared by the Expert Committee on Insecticides of WHO, and previously published in the reports of its sessions.

Specifications, with test procedures, are given for the following materials: technical DDT, technical benzene hexachloride, gamma-isomer BHC concentrates, technical methoxychlor, technical chlordan, technical dieldrin, and pyrethrum extract; DDT water dispersable powders and BHC water dispersable powders; and for emulsifiable concentrates of DDT, BHC and dieldrin. There follow specifications for compression sprayers, hand sprayers, and stirrup-pump-type sprayers. The omission of power sprayers should be remedied soon. Also, under compression sprayers, knapsack type, it is desirable to require a 2 inch depth top lip to minimize spillage on the operator. Specifications for hand dusters, both plunger and rotary types, are also presented, but nothing for power-operated dusters. In addition there are specifications for various accessories and parts, such as rubber hose, cut-off valves and hose connectors. There are also illustrations of certain specialized test equipment.

This is a very useful compilation of material of great value to manufacturers, vendors and purchasers of the insecticides covered. Within its present limitations it is highly recommended, but it is hoped that the Expert Committee will rapidly provide additional specifications for toxaphene and for the organic phosphate insecticides (e.g., EPN), for power-driven sprayers and dusters, for power-driven mist blowers, and for thermal and mechanical aerosol generators. Purchasers of the Manual will receive additions and amendments as they are issued.

HAROLD F. GRAY

Toxic Hazards of Certain Pesticides to Man, by J. M. BARNES, Division of Environmental Sanitation, WHO and Medical Research Council, Gr. Britain; WHO Monograph Series #16, 129 pp. and 53 pp. bibliography. Geneva, World Health Organization. Distributed by Columbia University Press, International Documents Service, 2960 Broadway, New York 27, New York, 1953. Price $1.50, Sw.fr. 6.—, Fr.fr. 480 (French edition in preparation).

This is a useful and concise statement of the toxicity of certain pesticides to man, in relation to their manufacture and application. The materials considered in some detail are DDT, aldrin, dieldrin, chlordan, BHC and gamma BHC, toxaphene, DDD (TDE) and methoxychlor. A section is devoted to the organo-phosphorus esters and amides such as TEPP and parathion, but there is little mention of EPN, which is proving to be valuable where DDT resistance has been encountered.

HAROLD F. GRAY

Joint FAO/WHO Expert Committee on Brucellosis; Second Report. By FAO and WHO. 34 pp. 1 Table. Geneva: WHO, Technical Report Series No. 67. May, 1953. (Issued also as FAO Agricultural Studies, No. 24). Available also in French edition. Price 2/—, Fr. fr. 80; Sw.fr. 1.—; $0.25.

This is a report of the second meeting of the Joint FAO/WHO Expert Committee on Brucellosis held in Italy in October, 1952. Appreciable advances in our knowledge of brucellosis have been made in the two years since the first report in epidemiology, diagnosis and prevention. The recommendations will be of interest to agricultural, veterinary and health authorities. The activities of the twelve FAO/WHO brucellosis centers situated all over the world are summarized, and the problem in its scientific and practical aspects is clearly stated. This will be of value both to the research worker and to those responsible for the control of animal and human brucellosis.

L. W. HACKETT

Expert Committee on Bilharziasis, First Report. 45 pp. Geneva: World Health Organization. Distributed by Columbia University Press, International Documents Service, 2960 Broadway, New York 27, New York. 2/3; $0.30, Sw.fr. 1.20

This report deals with the geographical distribution, diagnosis, epidemiology, survey methods, treatment and control of bilharziasis. The report is incomplete in respect to the distribution and epidemiology of *S. japonicum* infection; the Committee felt that more local studies are necessary before any definite pronouncement can be made on the role of domestic animals in its spread. While bilharziasis seems to be accepted by the Committee as the preferred name of the disease, the parasites are properly referred to as species of *Schistosoma*.

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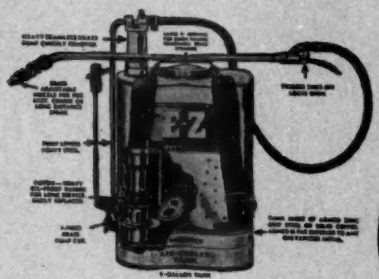

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